

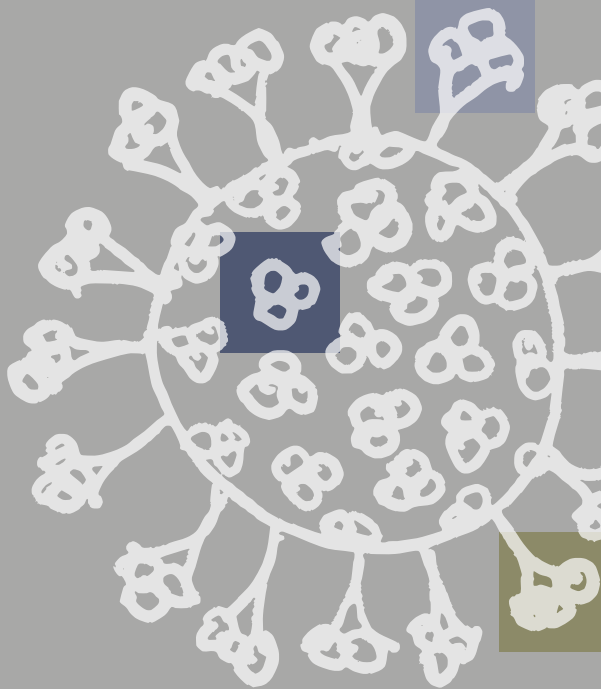


*Bernd Sebastian Kamps
Christian Hoffmann*

COVID REFERENCE

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Preface

Six weeks after the third edition, the world has changed again. The pandemic is raging in South America, particularly in Brazil, Ecuador and Peru. SARS-CoV-2 is under control in China, but in Iran it is not. And in Europe, where most countries have weathered the first wave and open borders to save a compromised tourist season, is now wondering if and for how long this biological *drôle de guerre* could last.

Science has moved ahead, too. We have seen a more complex picture of COVID-19 and new clinical syndromes; the first data from vaccine trials; first results from randomized controlled drug studies; encouraging publications on monoclonal neutralizing antibodies and serological evidence about the number of people who have come into contact with SARS-CoV-2. Unfortunately, we have also seen the first science scandal with fake data published in highly ranked journals. And we face new challenges like long-term effects of COVID-19 and a Kawasaki-like inflammatory multisystem syndrome in children.

For quite some time, prevention will continue to be the primary pillar of pandemic control. In future waves of the SARS-CoV-2 pandemic, we will focus on the conditions under which SARS-CoV-2 is best transmitted: crowded, closed (and noisy) places and spaces. Although hospitals are not noisy, they are crowded and closed, and the battle against the new coronavirus will be decided at the very center of our healthcare system. Over the next months and maybe years, one of all of our top priorities will be to give all healthcare workers and patients perfect personal protective equipment.

Bernd Sebastian Kamps & Christian Hoffmann

7 June 2020

Preface to the First Edition

Seventeen years ago, in the middle of the outbreak, we decided to write a short medical text about the ongoing SARS drama, presenting the scientific data and providing real-time updates. After publishing three editions in 6 months, a [scientific magazine](#) concluded that our *SARS Reference* (www.SARSReference.com) was “not fancy”, but presented “plenty of information”. When we became aware of the new coronavirus epidemic in mid-January 2020, we immediately felt that time had come to repeat our millenium exercise.

While SARS-CoV-2 seems under control in China, the epidemic is moving west briskly. What only weeks ago seemed an impossible feat – imposing and enforcing strict quarantine measures and isolating millions of people – is now a reality in many countries. People all over the world will have to adapt and invent new lifestyles in what is the most disruptive event since World War II.

We believe that the current situation needs a new type of textbook. Humanity is confronting an unknown and threatening disease which is often severe and fatal. Health care systems are overwhelmed. There is no proven treatment and vaccines will not be available soon. Such a situation has not existed since the flu pandemic in 1918.

We believe a clear head is crucial in times of over-information, with dozens of scientific papers published *every day*, news about hundreds of studies being planned or already on the way and social media blending hard data with rumors and fake news. The tedious work of screening the scientific literature and the scientific data has to be done – regularly & constantly, like a Swiss watch.

Over the coming months, COVID Reference will be presenting updates on a weekly basis and narrating the scientific data as coherently as possible.

Remember [Science Magazine](#). It isn't fancy.

[Bernd Sebastian Kamps](#) & [Christian Hoffmann](#)

29th March 2020

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0. Top 10

Please bookmark www.CovidReference.com/Top10Papers and come back at 19:00 CEST for the **Daily Top 10 Papers** on COVID-19. Each citation comes with a short comment and a link to the full-text article.

1. Epidemiology

Bernd Sebastian Kamps

Stefano Lazzari

In December 2019, several patients from Wuhan, People's Republic of China, developed pneumonia and respiratory failure reminiscent of the SARS epidemic in 2003 (WMHC 2019, www.SARSReference.com). In early January 2020, a new virus was isolated from bronchoalveolar lavage fluid samples and found to be a betacoronavirus (Zhou 2020). Between then and the time of this writing (7 June), the virus, later denominated SARS-CoV-2, has spread to every corner of the world. Millions have been diagnosed with SARS-CoV-2 infection and hundreds of thousands of people have died of COVID-19, the disease caused by SARS-CoV-2. SARS-CoV-2 has the potential to cause a long-lasting pandemic with high fatality rates.

In this chapter, we will discuss:

- Hotspots of SARS-CoV-2 infection
- The natural course of the COVID-19 pandemic and its mitigation by “lockdown” measures
- The effect of “lockdown” measures
- Special aspects of the pandemic in selected places
- A ‘COVID pass’
- The second wave

The transmission of SARS-CoV-2 is discussed in a separate chapter (page 71) which highlights that SARS-CoV-2 is easily transmissible both by symptomatic and asymptomatic individuals; it thrives in closed and densely inhabited environments; and is amplified by so-called ‘superspreader’ events. There is evidence

that in China, human-to-human transmission has occurred among close contacts since the middle of December 2019 (Li Q 2020). In Italy and France, SARS-CoV-2 was circulating as early as January among asymptomatic or paucisymptomatic people (Cereda 2020, Gámbaro 2020). In the Greater Paris Region, after retesting samples from 24 patients treated in December and January, one sample collected on December 27 was retrospectively found to be positive for COVID (France 24, 5 May 2020). The samples had initially been collected to detect flu using PCR tests.

The mean incubation period of SARS-CoV-2 infection is around 5 days (Li 2020, Lauer 2020, Nie X 2020). The serial interval – defined as the duration of time between a primary case-patient having symptom onset and a secondary case-patient having symptom onset – has been estimated to be between 5 and 7.5 days (Cereda 2020). SARS-CoV-2 is highly contagious, with an estimated basic reproduction number R_0 of around 2.5-3.0 (Chan 2020, Tang B 2020, Zhao 2020). [R_0 indicates the average number of infections one case can generate over the course of the infectious period in a naïve, uninfected population.]

Transmission Hotspots

The probability of SARS-CoV-2 transmission is a function of time and closeness of contact between infected and susceptible individuals. The following settings are catalyzers of local outbreaks:

- Homes (+ intense social life with friend and colleagues)
- Workplaces
- Hospitals
- Nursing facilities
- Cruise ships
- Aircraft carriers and other military vessels
- Mass gatherings and religious gatherings

- Schools
- Prisons
- Homeless shelters
- Industrial meat-packing plants
- Choirs

Homes

Infection rates at home varied widely (between 11% and 19%) in three studies (Bi Q 2020, Jing QL 2020, Li W 2020). One group noted that household contacts and those travelling with a COVID-19 case had a 6 to 7 times higher risk of infection than other close contacts, and that children were as likely to be infected as adults (Bi Q 2020). Another group found that the odds of infection among children and young people (<20 years old) was only 0.26 times of that among the elderly (≥ 60 years old) (Jing QL 2020). A third group calculated that the secondary attack rate in children was 4% compared to 17.1% in adults, and that the secondary attack rate in contacts who were spouses of index cases was 27.8% compared to 17.3% in other adult members in the households (Li W 2020). It has been objected that these transmission rates may be an underestimate if index cases were isolated outside of the home (Sun 2020). In yet another study, 32.4% (48 of 148) of household contacts of 35 index cases were infected (Wu J 2020); however, this percentage relied on the assumption that all secondary cases were infected by the index case. In single households, the transmission rates may probably reach 75% or more (Böhmer 2020).

Workplaces

As early as January 2020, SARS-CoV-2 was found to spread during workshops and company meetings ([Böhmer 2020](#)). Later, an outbreak of SARS-CoV-2 infection was reported from a call center where 94 out of 216 employees working on the same floor were infected, translating to an attack rate of 43.5% ([Park SY 2020](#)). Recently, outbreaks with hundreds of infected individuals were reported from meat-packing plants in Germany ([DER SPIEGEL](#)), the US ([The Guardian](#)) and France ([Le Monde](#)).

Particularly instructive is the case of a scientific advisory board meeting held in Munich, Germany, at the end of February. Eight dermatologists and 6 scientists (among them the index patient) met in a conference room of about 70 m² with a U-shaped set-up of tables separated by a central aisle >1 meter wide. During the meeting that lasted 9.5 hours, refreshments were served in the same room 4 times. In the evening, the participants had dinner in a nearby restaurant and shook hands for farewell, with a few short hugs (no kisses!). Finally, the index patient shared a taxi with three colleagues for about 45 min. The outcome: the index patient infected at least 11 of the 13 other participants. When isolated either in a hospital or at home these individuals infected an additional 14 persons ([Hijnen 2020](#)).

In the presence of an infected individual, workplaces can be important amplifiers of local outbreaks epidemics.

Hospitals and other health care centers

There is no doubt that transmission in hospitals and other health care centers (including doctors offices) played a prominent role in the origin and spread of local epidemics, especially in the beginning when suspicion of the disease was low. This is reminiscent of the largest MERS outbreak outside of the Arabian peninsula which occurred in the Republic of Korea in 2015. Of the 186

cases, 184 were infected nosocomially ([Korea Centers for Disease Control and Prevention 2015](#)).

Hospitals are a favorable environment for the propagation of SARS-CoV-2 ([Wison 2020](#)). In some instances, hospitals could have been even the main COVID-19 hub, as they were rapidly populated by infected patients, facilitating transmission to health workers and uninfected patients ([Nacoti 2020](#)). Within the first 6 weeks of the epidemic in China, 1,716 cases among health care workers were confirmed by nucleic acid testing, and at least 5 died (0.3%) ([Wu 2020](#)).

One hospital study reports that the virus was widely present in the air and on object surfaces in both the intensive care units and general wards, implying a potentially high infection risk for medical staff. Contamination was greater in ICUs. Virus was found on floors, computer mice, trash cans, sickbed handrails, and was detected in the air up to approximately 4 m from patients ([Guo 2020](#)). The virus was also isolated from toilet bowl and sink samples, suggesting that viral shedding in stool could be a potential route of transmission ([Young 2020](#), [Tang 2020](#)). However, most of these studies have evaluated only viral RNA. It remains to be seen whether this translates into infectious virus.

Although nosocomial spread of the virus is well documented, appropriate hospital infection control measures can prevent nosocomial transmission of SARS-CoV-2 ([Chen 2020](#)). This was nicely demonstrated by the case of a person in her 60s who travelled to Wuhan on Dec 25, 2019, returned to Illinois on Jan 13, 2020, and transmitted SARS-CoV-2 to her husband. Although both were hospitalized in the same facility and shared hundreds (n=348) of contacts with HCWs, nobody else became infected ([Ghinai 2020](#)).

However, working in a high-risk department, longer duty hours, and suboptimal hand hygiene after coming into contact with patients, are all associated with an increased risk of infection in

health care workers (Ran 2020). At one time, during the early epidemic in March 2020, around half of 200 cases in Sardinia were among hospital and other health care workers. On 14 April, the US CDC reported that 9,282 Health Care Personnel has been infected with SARS-CoV-2 in the USA.

The risk factors for SARS-CoV-2 infection in health care workers has recently been summarized in a review. There is evidence that more consistent and full use of recommended PPE measures was associated with decreased risk for infection, suggesting a dose-response relationship. Association was most consistent for masks but was also observed for gloves, gowns, and eye protection, as well as hand hygiene. Some evidence was found that N95 respirators might be associated with higher reduction of risk for infection than surgical masks. Evidence also indicated an association between certain exposures (such as involvement in intubations, direct contact with infected patients, or contact with bodily fluids) (Chou 2020).

SARS-CoV-2 outbreaks can occur everywhere, not only in admission, infectious disease and intensive care units. In a pediatric dialysis unit in Münster (Germany), a healthcare worker infected 7 colleagues, three young patients and one accompanying person (Schwierzeck 2020). A Chinese study of 9,684 healthcare workers (HCW) in Tongji Hospital confirmed a higher rate of infection in non-first-line HCW (93/6,574, 1.4%) when compared to those who worked in fever clinics or wards (17/3110, 0.5%) (Lai X 2020). Those who work in clinical departments other than fever clinics and wards may have neglected to adopt adequate protective measures.

In a well-documented report about nosocomial transmission recently published, a man sought help for coronavirus symptoms on March 9, spending only a few hours at the emergency department of a hospital in Durban, South Africa. He was kept separate in a triage area, but that room was reached through the

main resuscitation bay, where a stroke patient occupied a bed. Both patients were seen by the same doctor. After being infected, the stroke patient caused a chain of transmission with 39 patients and 80 staff in 16 different departments being infected, and 15 patients dying. The study found that patients infected few other patients directly. Instead staff members spread the disease from patient to patient and from department to department, perhaps sometimes without becoming infected themselves (Nordling 2020). Strictly enforcing infection control measures and screening hospital staff will be important measures in future waves of COVID-19.

Long-term care facilities

Long-term care facilities are high-risk settings for infectious respiratory diseases. In a skilled nursing facility in King County, Washington, US, 167 cases of COVID-19 were diagnosed within less than three weeks from the identification of the first case: 101 residents, 50 health care personnel and 16 visitors (McMichael 2020) (Table 1).

Among residents (median age: 83 years), the case fatality rate was 33.7%. Chronic underlying conditions included hypertension, cardiac disease, renal disease, diabetes mellitus, obesity, and pulmonary disease. The study demonstrates that once introduced in a long-term care facility, often by a care worker or a visitor, SARS-CoV-2 has the potential to spread rapidly and widely, with devastating consequences.

A national survey covering 96% of all long-term care facilities in Italy found that in Lombardy, the epicenter of the epidemic, 53.4% of the 3,045 residents who died between 1 February and 14 April were either diagnosed with COVID-19 or presented flu-like symptoms, a death rate among residents of 6.7%. Among the 661 residents who were hospitalized during the same period, 199 (30%) were found positive by a PCR test. According to WHO esti-

mates, in countries in the European Region up to half of those who have died from COVID-19 were residents in long-term care facilities (see the statement to the press by [Hans Henri P. Kluge, WHO Regional Director for Europe](#)). Excess mortality data suggests that in several countries many deaths in long-term care facilities might have occurred in patients not tested for COVID-19, which are often not included in the official national mortality statistics.

Table 1. COVID outbreak in a long-term care facility

	Residents (N = 101)	Healthcare personnel (N = 50)	Visitors (N = 16)
Median age (range)	83 (51-100)	43.5 (21-79)	62.5 (52-88)
Female (%)	68.3	76	31.2
Hospitalized (%)	54.5	6.0	50.0
Died (%)	33.7	0	6.2
Chronic underlying conditions (%)			
Hypertension	67.3	8.0	12.5
Cardiac disease	60.4	8.0	18.8
Renal disease	40.6	0	12.5
Diabetes mellitus	31.7	10.0	6.2
Obesity	30.7	6.0	18.8
Pulmonary disease	31.7	4.0	12.5

SARS-CoV-2 continues to spread in US nursing homes where approximately 1.3 million Americans reside ([CDC 200311](#)). In mid-April, more than 1,300 facilities had identified infected patients ([Cenziper 2020](#)). As most residents have one or more chronic underlying condition such as hypertension, cardiac disease, renal disease, diabetes mellitus, obesity and pulmonary disease, COVID-19 puts them at high risk for premature death.

Cruise ships

Cruise ships carry many people in confined spaces. On 3 February 2020, 10 cases of COVID-19 were reported on the Diamond Princess cruise ship. Within 24 hours, all sick passengers were isolated and removed from the ship and the rest of the passengers quarantined on board. Over time, more than 700 of 3,700 passengers and crew tested positive (around 20%). One study suggested that without any intervention 2,920 individuals out of the 3,700 (79%) would have been infected ([Rocklov 2020](#)). The study also showed that an early evacuation of all passengers on 3 February would have been associated with only 76 infected.

For cruise ships, SARS-CoV-2 may spell disaster as carrying village-loads of people from one place to another may not be a viable business model until the global availability of a safe and efficient vaccine.

Aircraft carriers and other military vessels

Big navy vessels such as aircraft carriers can become floating petri dishes for emerging viral respiratory diseases. Already in 1996, an outbreak of influenza A (H3N2) occurred aboard a navy ship. At least 42% of the crew became ill within few days, although 95% had been appropriately vaccinated ([Earhart 2001](#)). Since the beginning of the year, several outbreaks of COVID-19 on military ships have been reported, facilitated by the small enclosed areas of work and the lack of private quarters for the crew. The largest outbreaks have been reported on the USS *Theodore Roosevelt* and the French aircraft carrier *Charles de Gaulle*. During the Theodore Roosevelt outbreak in late March, around 600 sailors out of a crew of 4,800 were infected with SARS-CoV-2 (see also the March 30 entry of the Timeline); around 60% remained asymptomatic. One active duty sailor died ([USNI News](#)). On the French aircraft carrier *Charles-de-Gaulle*, a massive epidemic was confirmed on 17 April. Among the 1,760 sailors, 1,046

(59%) were positive for SARS-CoV-2, 500 (28%) presented symptoms, 24 (1.3%) sailors were hospitalized, 8 on oxygen therapy and one in intensive care.

Smaller clusters have also been reported on 5 other US military vessels, and in one each from France, Taiwan, and Holland. However, given usual security policies and communication restrictions of national armies and navies, it is possible that other unreported cluster of cases and even deaths might have occurred.

For aircraft carriers, the potential for further outbreaks at any time might well interfere with full operability.

Mass gatherings

Several mass gathering events have been associated with explosive outbreaks of COVID-19. As of April 24, 2020, a total of 5,212 coronavirus cases were related to an outbreak at the Shincheonji Church in South Korea, accounting for about 48.7% of all infections in the country.

A football match played in Milan, Italy on 19 February 2020 has been described as “Game zero” or “a biological bomb”. The match was attended by 40,000 fans from Bergamo and 2,500 from Valencia and played just two days before the first positive case of COVID-19 was confirmed in Italy. 35 percent of Valencia’s team members tested positive for the coronavirus a few weeks later, as did several Valencia fans. By mid-March, there were nearly 7,000 people in Bergamo who had tested positive for the coronavirus with more than 1,000 deaths, making Bergamo the most heavily hit province during the COVID-19 outbreak in Italy. Valencia also had 2,600 cases of the infection.

The annual gathering of the Christian Open Door Church held between 17 and 24 February in Mulhouse, France, was attended by about 2,500 people and became the first significant cluster in

France. After a parishioner and 18 family members tested positive on 1 March, a flurry of reported cases brought the existence of a cluster to light. According to an investigative report by France Info, more than 1,000 infected members from the rally in Mulhouse contributed to the start of the COVID-19 epidemic in France. A large number of diagnosed cases and deaths in France as well as Switzerland, Belgium and Germany were linked to this gathering.

Religious celebrations

One report describes 35 confirmed COVID-19 cases among 92 attendees at church events during March 6–11. The estimated attack rates ranged from 38% to 78% ([James 2020](#)). In Frankfurt, Germany, one of the first post-lockdown clusters started during a religious ceremony held on 10 May. As of 26 May, 112 individuals were confirmed to be infected with SARS-CoV-2 ([Frankfurter Rundschau](#)).

The bottom line: Going to church does not protect from SARS-CoV-2.

Schools and schoolchildren

Schoolchildren usually play a major role in the spread of respiratory viruses, including influenza. However, while the SARS-CoV-2 virus has been detected in many children, they generally experience milder symptoms than adults, need intensive care less frequently and have a low death rate.

The possible role of children in SARS-CoV-2 transmission is still unclear. In a small COVID-19 cluster detected in the French Alps at the end of January, one person returning from China infected eleven other people, including a nine-year-old schoolboy. The researchers closely tracked and tested all contacts ([Danis 2020](#)). The boy had gone to school after showing COVID-19 symptoms and was estimated to have had more than sixty high-risk close

contacts. No one was found positive to the coronavirus, though many had other respiratory infections. Also, no virus was found in the boy's two siblings who were on the same Alpine vacation. The researchers concluded that "because children are less likely to become infected and symptoms are milder, they may play a less important role in the spread of the new virus".

A Norwegian Institute of Public Health review of the role of children in the transmission of SARS-CoV-2 found five documented cases of likely spread of disease from children, but concluded that the evidence is sparse and it is too early to say if children play an important role in the spread of the disease ([Fretheim 2020](#)). However, a pre-print study of SARS-CoV-2 viral load by patient age conducted by the Institute of Virology, Charité-Universitätsmedizin Berlin, did not find any statistical difference in viral load in different age groups, concluding that children may be as infectious as adults and suggesting to use caution in the re-opening of schools and kindergartens in the present situation ([Jones 2020](#)). The debate continues.

Prisons

According to the WHO, people deprived of their liberty, such as people in prisons and other places of detention, are more vulnerable to the coronavirus disease (COVID-19) outbreak ([WHO 200315](#)). People in prison are forced to live in close proximity and thus may act as a source of infection, amplification and spread of infectious diseases within and beyond prisons. The global prison population is estimated at 11 million and prisons are in no way "equipped" to deal with COVID-19 ([Burki 2020](#)). The UN High Commissioner for Human Rights, Michelle Bachet, has encouraged governments to release inmates who are especially vulnerable to COVID-19, such as older people, as well as low-risk offenders, and a number of countries are taking action to try to reduce the prison population.

As of 21 April, SARS-CoV-2 was present in US correctional and detention facilities. Aggregated data on cases from 37 of 54 state and territorial health department jurisdictions revealed 4,893 cases and 88 deaths among incarcerated and detained persons and 2,778 cases and 15 deaths among staff members ([Wallace 2020](#)).

Homeless shelters

Testing in 1,192 residents and 313 staff members in 19 homeless shelters from 4 US cities ([see table](#)), initially triggered by the identification of a COVID-19 cluster, found infection rates of up to 66% ([Mosites 2020](#)).

In another report from Boston, Massachusetts, 147/408 (36%) homeless shelter residents were positive. Of note, 88% had no fever or other symptoms at the time of diagnosis ([Baggett 2020](#)).

Industrial meat-packing plants

On 5 May 2020, the German magazine [DER SPIEGEL](#) reported that more than 600 employees were infected with SARS-CoV-2 at meat processing plants in Germany. One week later, [The Guardian](#) reported that almost half of the current COVID-19 hotspots in the US were linked to meat processing plants where poultry, pigs and cattle are slaughtered and packaged. At the same time, around a hundred people tested positive in two meat processing plants in France ([Le Monde](#)).

Promiscuity, cold and humid conditions are currently favored as explanations for these unusual outbreaks.

Choirs

On 8 March 2020, the Amsterdam Mixed Choir gave a performance of Bach's St John Passion in the city's Concertgebouw Auditorium. Days later, the first singers developed symptoms and

in the end 102 of 130 choristers were confirmed to have COVID-19. One 78-year-old choir member died, as did three colleagues; some singers required intensive care ([The Guardian, 17 May](#)).

On 9 March, members of the Berlin Cathedral Choir meet for their weekly rehearsal. Three weeks later, 32 out of 74 choir members were positive for SARS-CoV-2 ([NDR 2020](#)). All recovered.

On 10 March 2020, 61 members of a Skagit County, Washington, choir met for a 2.5-hour practice. A few weeks later, researchers reported that 32 confirmed and 20 probable secondary COVID-19 cases had occurred (attack rate = 53.3% to 86.7%); three patients were hospitalized, and two died. The authors conclude that transmission was likely facilitated by close proximity (within 6 feet) during practice and increased virus diffusion by the act of singing ([Hamner 2020](#)).

These data suggest that any noisy, closed and stagnant air environments (e.g, discos, pubs, birthday parties, restaurants, butchering facilities, etc.) where people stand, sit or lie close together and are required to shout for communication are ideal conditions for generating large SARS-CoV-2 outbreaks.

SARS-COV-2 re-activation or re-infection?

In South Korea and elsewhere more than 100 people who had recovered from COVID-19 were retested positive ([Ye 2020](#)) and there was concern that patients who recover from COVID-19 may be at risk of reinfection. However, there was no indication that they were contagious. The most likely explanation is that the ‘infection had been reactivated’ in the patients or that the tests picked up non-infective RNA of the virus. Very preliminary data from an animal study (n=2) suggest that that immunity acquired following primary infection may protect upon subsequent exposure to the virus. Infection of rhesus macaques with SARS-CoV-2 and re-infection after recovery showed that there was no viral

replication in nasopharyngeal or anal swabs, nor any other signs of COVID-19 disease recurrence ([Bao 2020](#)).

Blood Transfusion

After screening 2,430 donations (1,656 platelet and 774 whole blood) with real-time PCR, authors from Wuhan only found plasma samples positive for viral RNA from 4 asymptomatic donors ([Chang 2020](#)). It remains unclear whether detectable RNA signifies infectivity. A preliminary report of a study in Dutch blood donors found that in April 2020 around 3% had detectable antibodies against SARS-CoV-2 ([NLTimes.nl](#)).

In a Korean study, seven asymptomatic blood donors were later identified as COVID-19 cases. None of 9 recipients of platelets or red blood cell transfusions tested positive for SARS-CoV-2 RNA ([Kwon 2020](#)). However, more data are still needed before we can conclude that transmission through transfusion is unlikely.

The pandemic

Natural course of a pandemic

The COVID-19 epidemic started in Wuhan, in Hubei province, China, and spread within 30 days from Hubei to the rest of mainland China, to neighboring countries (in particular, South Korea, Hong Kong and Singapore) and west to Iran, Europe and the American continent. The first huge outbreaks occurred in regions with cold winters (Wuhan, Iran, Northern Italy, the Alsace region in France).

Fifty years ago, the course of the COVID-19 pandemic would have been quite different, with slower global spread but high burden due to limited diagnostic and therapeutic capacities and no option of nation-wide lockdowns (see also a report of the influenza pandemics in 1957 and 1968: [Honigsbaum 2020](#)). According to one (controversial) simulation, in the absence of interventions

and with a mortality rate of around 0.5%, without interventions COVID-19 would have resulted in 7.0 billion infections and 40 million deaths globally during the first year ([Patrick 2020](#)). The peak in mortality (daily deaths) would have been observed approximately 3 months after the beginning of local epidemics. Another model predicted that 80% of the US population (around 260 million people) would have contracted the disease. Of those, 2.2 million Americans would have died, including 4% to 8% of those over age 70 ([Ferguson 2020](#)).

Despite these dire predictions, some epidemiologists and senior politicians seriously considered implementing only limited mitigating measures, based on two questionable arguments:

- The country would not have to face the dramatic economic downturn that seems unavoidable in countries and states which opted for strict containment measures (China, Italy, Spain, France, California, New York, to name a few). However, most economists would argue that there would still be an economic downturn due to self-imposed restrictions by the population and businesses, as shown by the major economic impact even in countries with less severe restrictions (e.g., [Sweden](#)).
- After a few months, up to 70% of the population could be naturally immunized (through infection with SARS-CoV-2) and protected against further outbreaks, able to look ahead to the next winter season with an even temper. (However, it is still unclear how long such acquired immunity would last? Maybe only a few months or few years? See the *Immunology* chapter, page 125).

In mid-March 2020, the prime minister of a former EU country proposed the approach of ‘letting the virus spread until we reach herd immunity’ as the best solution to the epidemic his nation was about to face. The shock treatment: accepting that a large

majority of the population would contract the virus, thus developing a collective immunity and preventing future coronavirus epidemics. The estimated figures from simulation models were dire. With a little over 66 million inhabitants, some 40 million people would have been infected, 4 to 6 million could have become seriously ill, 2 million requiring intensive care. Around 400,000 Britons may have died. The prime minister stated: “Many more families are going to lose loved ones before their time.” Faced with the rapid increase of cases and deaths and a public uproar, the PM eventually made a U-turn, implementing strict confinement measures as other countries were doing.

Only one European country, Sweden, has decided to pursue a strategy of limited public health measures (e.g., protection of older age groups, widespread testing, individual social distancing measures) without enforcing strict rules of confinement or business shutdowns. The results will be briefly discussed below on page 51.

Pandemic 2.0: Lockdowns

Fortunately, for now, the world has been spared from a freely circulating SARS-CoV-2. If humanity can change the climate, why shouldn't we be able to change the course of a pandemic? Although economists warned that **unemployment** could surpass the levels reached during the **Great Depression in the 1930s**, at first, almost all governments considered saving hundreds of thousands lives more important than avoiding a massive economic recession. First in China, six weeks later in Italy and another a week later in most Western European countries, more recently in the US and in many other countries in the world, unprecedented experiments of gigantic dimensions were started: ordering entire regions or the whole nation to lockdown. In Italy and Spain, people were ordered to stay home, except for conducting “essential activities” (i.e., purchasing food, medicines,

and other basic supplies, going to hospital, or performing essential work). Italians were told to stay at home even on the popular *Pasquetta* day, Easter Monday, where people usually flock to the seaside or countryside to enjoy a picnic with family and friends. Eventually, Italians were even restricted from moving from one municipality to another.

While there are differences in the implementation of the lockdown from one country to another, some common measures include:

- Restrictions of movement from home, unless it is strictly necessary (confinement or “stay-at-home” order)
- Ban on all public mass gatherings, including concerts, festivals, rallies, even religious events ([Tian H 2020](#))
- Closure of schools and universities
- Closure of all retail shops, except for those serving primary needs (food, medicines, gas stations, newsstands, etc)
- Shutting down of all industries and factories, except where providing essential products
- Border closing with neighbouring countries, international travel bans. In some cases, restrictions of travel within the country outside the area or region of residence.

Lockdowns have been used in the past to control disease outbreaks, usually in limited areas and for limited periods. China was the first country to implement, on 23 January, a strict and total lockdown in a city of 11 million people, later extended to the whole Hubei province (WHO called this “unprecedented in public health history”). The lockdown lasted 2 months.

Italy was the first country to implement a nationwide lockdown to the whole country on 9 March, to be followed by Denmark (11

March), Ireland and Norway (12 March), Spain and Poland (13 March), Switzerland, France, Belgium (17 March) and then most other European countries. By 26 March, 1.7 billion people worldwide were under some form of lockdown, which increased to 3.9 billion people by the first week of April – more than half of the world's population. Lockdowns in Europe were generally less strict than in China, allowing the continuation of essential services and industries and the circulation of people when justified.

Lockdown outcomes

The expected result of lockdown measures is the breaking of the chain of SARS-CoV-2 transmission, leading to a reduction of the number of new infections, hospitalization and ultimately deaths. This can be measured in different ways, including by the number of

- SARS-CoV-2 newly infected people
- Hospital admissions for COVID-19
- Patients treated in intensive care units (ICU)
- Deaths

Number of infections

Figure 1 proved as early as four weeks after the Wuhan lockdown that strict containment measures are capable of curbing a SARS-CoV-2 epidemic. The figure presents the Chinese COVID-19 epidemic curves of laboratory-confirmed cases, by symptom onset (blue) and – separately – by date of report (orange). The data were compiled on 20 February 2020, four weeks after the beginning of the containment measures which included a lockdown on nearly 60 million people in Hubei province as well as travel restrictions for hundreds of millions of Chinese citizens. The blue

columns show that (1) the epidemic rapidly grew from 10-22 January, (2) reported cases (by date of onset) peaked and plateaued between 23 January and 28 January and (3) steadily declined thereafter (apart from a spike reported on 1 February). Based on these data, we would now expect a decline in reported cases around three weeks after a general lockdown.

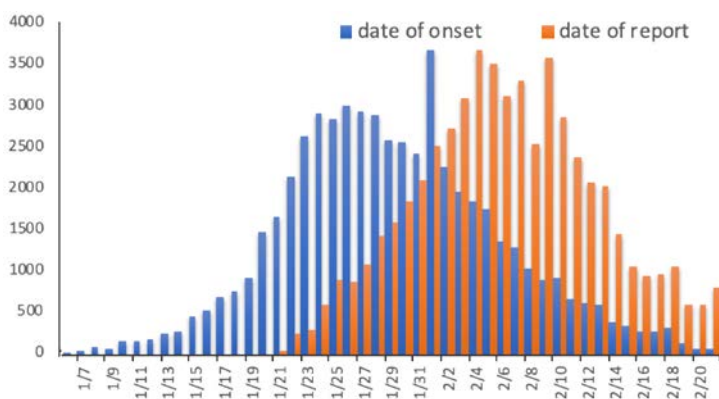


Figure 1. The Chinese outbreak in January/February 2020. Epidemic curves by symptom onset and date of report on 20 February 2020 for laboratory confirmed COVID-19 cases for all of China. Modified from *Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19)*, 16-24 February 2020. [https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-\(covid-19\)](https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19))

However, the number of newly diagnosed SARS-CoV-2 cases is of limited usefulness since, being closely related to the number of tests being performed, do *not reflect the true number of infections that have occurred*. To know the true number, the entire population would need to be tested repeatedly which is, of course, impractical. PCR tests are usually performed in symptomatic patients or, in some cases, in close contacts and most asymptomatic cases will be missed. Seroprevalence studies in population

samples that are being implemented can provide a better estimate of the number of people who have been infected in the past but will not directly measure the incidence (new infections). Best incidence estimates can only be made by mathematical modelling. Not surprisingly, the first models of the European epidemic revealed that reported COVID-19 cases represent only a fraction of those truly infected. A model based on observed deaths in 11 European countries suggested that true infections were much higher than reported cases (Flaxman 2020). According to the model, as of 28 March, 5.9 million people in Italy and 7 million in Spain could have been SARS-CoV-2-infected (Table 2). Germany, Austria, Denmark, and Norway would have the lowest attack rates (proportion of the population infected). If these assumptions are validated, the true number of cases would outnumber the reported cases on March 28 (Italy: 92,472; Spain: 73,235; France: 37,575) by up to two orders of magnitude

[The data provided by Flaxman et al. immediately invited us in March to do some *kitchen epidemiology*. First: if on 28 March the number of infected people in Italy was around 6 million (with a credible interval of 2 to 15 million) and if we assumed that 18 days later the total number of deaths in Italy was around 30,000 (the official figure reported on 15 April was 21,645 deaths), the mortality of COVID-19 infection in Italy could be in the range of 0.5% (0.19%-1.6%).

Table 2. Estimates of total population infected as of 28 March 2020

Country <i>Deaths on 28 March</i>	% of population infected*	Population infected*
Austria 68	1.1% (0.36%-3.1%)	96,800 (31,680-272,800)
Belgium 353	3.7% (1.3%-9.7%)	425,500 (149,500-1,115,500)
Denmark 65	1.1% (0.40%-3.1%)	63,800 (23,200-179,800)
France 2,314	3.0% (1.1%-7.4%)	2,010,000 (737,000-4,958,000)
Germany 433	0.2% (0.28%-1.8%)	166,000 (232,400-1,494,000)
Italy 10,023	9.8% (3.2%-26%)	5,919,200 (1,932,800-15,704,000)
Norway 23	0.41% (0.09%-1.2%)	21,600 (4,860-64,800)
Spain 5,982	15% (3.7%-41%)	7,035,000 (1,735,300-19,229,000)
Sweden 105	3.1% (0.85%-8.4%)	316,200 (86,700-856,800)
Switzerland 264	3.2% (1.3%-7.6%)	275,200 (111,800-653,600)
UK 1,019	2.7% (1.2%-5.4%)	1,798,200 (799,200-3,596,400)

*mean (95% credible interval)

Data source: Flaxman S et al. (Imperial College COVID-19 Response Team). Report 13: Estimating the number of infections and the impact of non-pharmaceutical interventions on COVID-19 in 11 European countries. 30 March 2020. DOI: <https://doi.org/10.25561/77731>

Second: if at the end of March, around 60% of all deaths in Italy were reported from Lombardy, 60% of the 6 million projected Italian SARS-CoV-2 infections – 3.6 million – would have occurred in a region with a population of 10 million. Moreover,

20% of all deaths in Italy were reported from the province of Bergamo alone which has a population of one 1.1 million.]

Seroprevalence studies underway in several European countries and in the US will clarify these figures soon. Preliminary results of a population survey in Los Angeles County released by USC on 20 April that tested 863 adults found that approximately 4.1% had antibodies to the virus ([USC News, 20 April 2020](#)). Adjusting for the statistical margin of error it suggested that between 2.8% to 5.6% of the county's adult population, approximately 221,000 to 442,000 adults, had been infected. That estimate is 28 to 55 times higher than the 7,994 confirmed cases of COVID-19 reported to the county at the time of the study. The number of COVID-related deaths in the county had then surpassed 600. On 13 May, [preliminary results](#) from a nationwide coronavirus antibody study showed that about 5% of the overall Spanish population had contracted the virus, with spikes in prevalence of 11.3% in Madrid and 14.2% and 13.5% in the central regions of Castilla y Leon and Castilla La Mancha. That is about 10 times more than the number of diagnosed cases.

Hospital admissions for COVID-19

Hospitalizations for COVID-19 are usually recorded and reported as part of the regular health care monitoring system. Several countries are regularly reporting the daily number of COVID-19 hospital admissions as an indicator of the trend in the epidemic. The advantage of monitoring hospital admission is that it can detect changes in transmission dynamics more quickly than the more lagged measures of (incidence of) ICU admissions and deaths (mortality rates). However, hospital admissions have limitations (hospital admission criteria may change from place to place and be modified over time) and can be influenced by, for example, the availability of quality home-based care or health system collapse. In addition, many governments are not publicly

providing numbers of daily hospital admissions and discharges (Garcia-Basteiro 2020).

Admissions into intensive care units

A more reliable indicator of the epidemic trend is the number of people treated in intensive care units. In France, the number of new hospital ICU admissions peaked on 1 April (Figure 2), while the daily variation in people treated in ICU (the balance between ICU entries and exits; Figure 3) started being negative one week later. However, this indicator can be influenced by the number of ICU beds and trained health personnel available for COVID-19 patients. If overwhelmed, hospitals might be forced to limit the admission to patients with more chances of survival, or patients might die at home (Grasselli 2020). In most developing countries, the very small number of ICUs will make this indicator of limited use.

Figure 4 shows the daily number of COVID-19 patients treated in ICU units in France.

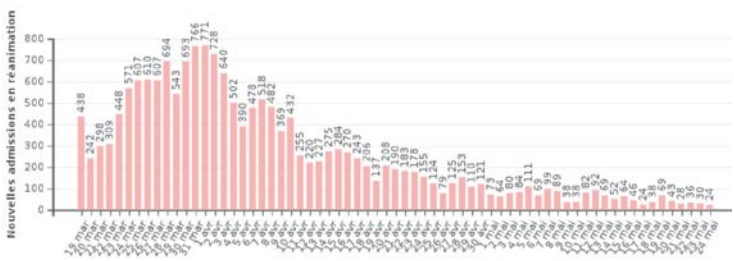


Figure 2. Daily number of new hospital ICU admissions for COVID-19 (y-axis: *Nouvelles admissions en réanimation*).

Source: [Pandémie de Covid-19 en France](#), Wikipedia.

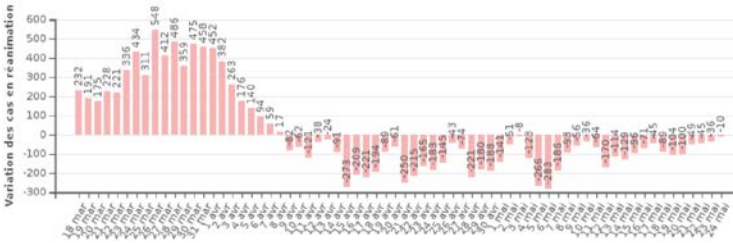


Figure 3. Daily variation in the number of people in ICU for COVID-19 (y-axis: *Variation des cas en réanimation*).

Source: [Pandémie de Covid-19 en France](#), Wikipedia.

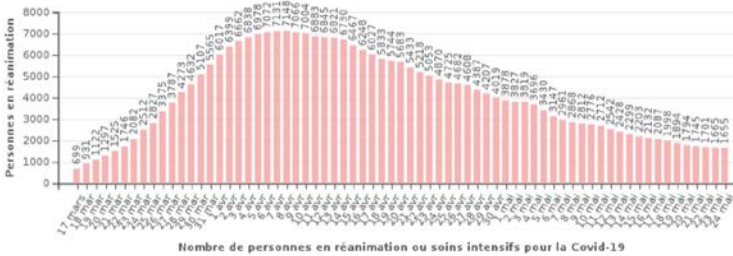


Figure 4. Daily number of COVID-19 patients in ICU units (y-axis: *Personnes en réanimation*).

Source: [Pandémie de Covid-19 en France](#), Wikipedia.

Deaths

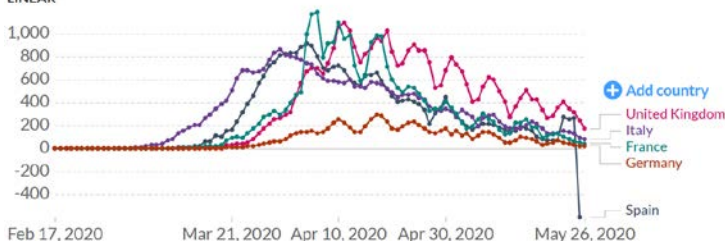
Asymptomatic infections go unnoticed; even mild to moderate symptoms may go unnoticed; deaths do not. Consequently, deaths reflect the reality of the COVID-19 epidemic better than the number of SARS-CoV-2-infected people. They will, however, only provide a picture of the number of infections that have occurred 2-4 weeks before (given the median incubation period and the period of hospitalization).

However, the current numbers of COVID-19 deaths are incomplete and will soon need to be corrected upwards. (By 10%, 30%, 50% or more? Nobody knows yet.) In Italy, especially in the most hit Northern regions, a certain number of people died at home and did not appear in the official reports. Data about overall mortality in epidemic hot spots in [Northern Italy \(ISS 2020\)](#) and in [Spain \(Madrid\)](#) suggests that excess mortality due to COVID-19 might be twice the officially reported figure. In France and the UK, as in other countries, deaths from long-term care facilities were initially not included in the official data. Figure 2 shows that the number of daily deaths decreases about three weeks after the implementation of lockdown measures (Italy: 8/10 March; Spain: 14 March).

Daily confirmed COVID-19 deaths, rolling 3-day average

Limited testing and challenges in the attribution of the cause of death means that the number of confirmed deaths may not be an accurate count of the true number of deaths from COVID-19.

LINEAR



Source: European CDC – Situation Update Worldwide – Last updated 26th May, 11:00 (London time)

Note: The rolling average is the average across three days – the confirmed deaths on the particular date, and the two previous days. For example, the value for 27th March is the average over the 25th, 26th and 27th March.

OurWorldInData.org/coronavirus • CC BY

Figure 5. Daily confirmed COVID-19 deaths, rolling 3-day average. Source: www.ourworldindata.org

The data from Europe show that lockdown measures were effective but less so than in China, probably reflecting a less strict lockdown in Europe. Daily updates are available from www.ourworldindata.org (Figure 5).

To calculate COVID-19 excess mortality over 1 year, based on age, sex, and underlying condition-specific estimates, an online tool is now available ([OurRisk.CoV](#)). For the UK, 293,991 deaths would be expected in a “do-nothing scenario”. With mitigation (i.e., less rigorous and voluntary measures), authors predicted between 18,000 and 37,000 deaths ([Banerjee 2020](#)).

Special Aspects of the Pandemic

The COVID-19 pandemic has highlighted a number of specific aspects and lessons learned that should be kept in mind during the management of future pandemics (by coronaviruses, influenza viruses or by as yet unknown viruses):

- First outbreak (China)
- Surprise or unpreparedness (Italy)
- Unwillingness to prepare (UK, USA, Brazil)
- Partial preparedness (France)
- Preparedness (Germany)
- Herd immunity (Sweden)
- Deferred beginning (South America)
- Splendid isolation (New Zealand, Australia)
- Unknown outcome (Africa)

First outbreak (China)

China was caught by surprise of the COVID-19 outbreak – as any other nation would have been – but “thanks” to the SARS outbreak in 2003 ([Kamps-Hoffmann 2003](#)), was prepared for it. At first, the epidemic spread within Wuhan and Hubei Province (December 2019) and then nationwide to all provinces in January 2020, favored by travelers departing from Wuhan before the Chinese Spring Festival ([Zhong 2020](#), [Jia JS 2020](#)). However, with-

in 3 weeks from the identification of the identification of a new virus, the government ordered the lockdown of more than 50 million in Wuhan and the surrounding province Hubei as well as travel restrictions for hundreds of millions of Chinese citizens. This astonishing first in human history achieved what even specialists didn't dare dream: curbing an epidemic caused by a highly contagious virus (Lau 2020).

As early as four weeks after the Wuhan lockdown, there was evidence that strict containment measures were capable of curbing a SARS-CoV-2 epidemic as demonstrated in Figure 1 (page 38). The lesson from China: it is possible to lockdown entire provinces or countries and lockdown works. Some authorities in the Western Hemisphere followed the example of China (Italy, for example, ordered a lockdown as early as 18 days after the diagnosis of the first autoctonous case), other governments didn't.

Preparedness (Taiwan)

On 7 June, **Taiwan** (24 million people with a population density of 650/km²), had reported 443 cases and 7 deaths. Most SARS-CoV-2 infections were not autochthonous. As of 6 April 2020, 321 cases were imported by Taiwanese citizens who had travelled once or more to 37 countries for tourism, business, work, or study (Liu JY 2020). From the beginning, Taiwan drew on its SARS experience to focus on protecting health care worker safety and strengthening the pandemic response (Schwartz 2020 + The Guardian, 13 March 2020). An early study suggested that identifying and isolating symptomatic patients alone might not suffice to contain the epidemic and recommended more generalized measures such as social distancing (Cheng HY 2020). Big data analytics were used in containing the epidemic. On one occasion, authorities offered self-monitoring and self-quarantine to 627,386 persons who potentially had contact with the more than 3000 passengers of a cruise ship. These passengers had disem-

barked at Keelung Harbor in Taiwan for a 1-day tour five days before the COVID-19 outbreak on the Diamond Princess cruise ship on February 5, 2020 ([Chen CM 2020](#)).

At the time of this writing, Taiwan is definitely one of the countries with the most successful management of COVID-19.

Surprise or unpreparedness (Italy)

Italy was the first European country struck by the pandemic. Complete genome analysis of SARS-CoV-2 isolates suggests that the virus was introduced on multiple occasions ([Giovanetti 2020](#)). Although the first local case was diagnosed only on 20 January, the force of the outbreak also suggests that the virus had been circulating for weeks, possibly as early as 1 January ([Cereda 2020](#)).

However, it was not straightforward to decipher the subtle signs of coming events. During the yearly flu season, COVID-19 deaths in elderly people could easily be interpreted as flu deaths. On the other end of the age spectrum, among the most active social age group – young people crowded in bars, restaurants and discos –, the rapid SARS-CoV-2 virus would not have caused life-threatening symptoms. Before being detected, the epidemic had time (at least a month) to grow.

There is one additional possible reason for the delay in recognizing the encroaching epidemic in Italy that is worth mentioning: the Italian ‘suspected case definition for COVID-19’. It included (like the suspected [case definitions recommended at that time by WHO](#)) the mandatory epidemiological criteria of ‘history of travel to China or in contact with a person from China’ before requesting a PCR test. A strict application of this case definition discouraged testing suspected pneumonia cases where the link with China was not clear (which would eventually happen everywhere after the first asymptomatic individuals had spread the infection). The anesthesiologist who eventually requested the

PCR test for Italian patient #1, Mattia, did it “[under her own responsibility and not in line with MOH guidelines](#)”.

It is as yet unclear why the epidemic took such a dramatic turn in the northern part of Italy, especially in Lombardy ([Gedi Visual 2020](#)), while other areas, especially the southern provinces, were relative spared. Of note, healthcare in Italy is run by the regions and for a long time, the Lombardy Region has favored the development of a mostly private and hospital-centered system, with great facilities but poor community-based services. This meant that patients were quickly run to the hospital, even those with minor symptoms, resulting in overcrowded emergency services and major nosocomial spread. A more decentralized and community-based system like in the [Veneto Region](#) (plus maybe a bit of luck) could have greatly reduced the mortality from COVID-19 in Lombardy. In addition, Italy had not updated nor implemented the 2006 national pandemic preparedness plan (<https://www.saluteinternazionale.info/2020/04/cera-una-volta-il-piano-pandemico>). The lack of preparedness and the overlap of responsibilities hampered considerably the initial coordination of the national response between the regions and the central government.

Unwillingness to prepare/denial (UK, USA, Brazil)

In the UK, clumsy political maneuvering delayed the start of effective lockdown measures by a week or more. As the epidemic doubles in size about every 7 days ([Li 2020](#)), around 50% and 75% of all deaths might have been prevented had lockdown or social distancing measures been ordered one or two weeks earlier, respectively. Early data from [Ireland and the United Kingdom](#) seem to confirm this assumption.

Like in Iran, where the regime covered up news of the coronavirus for three days to avoid impacting turnout at parliamentary elections on 21 February, domestic politics (or paranoia; [BMJ](#),

6 March 2020) influenced the epidemic response in the United States of America. Scientific advice from CDC and other national public health institution was ignored ([The Lancet 2020](#)). The US is now the country with the highest number of cases and deaths (2 million and more than 110,000 on 7 June, respectively). Without this unprecedented vacuum in leadership in US, most of these deaths would have been prevented.

Brazil, which is also not an example of good governance performance, is on track to be the country with the second highest number of deaths.

Partial preparedness (France)

France was partially prepared, partially not. During the first national outbreak near Mulhouse, hospitals were overwhelmed. Despite the updated and well structured pandemic plan (<https://www.gouvernement.fr/risques/plan-pandemie-grippale>), all over the country protective equipment was in short supply; in particular, face masks were sorely lacking after a decision of the Hollande government to greatly reduce the expensive stocks of 1.7 billion protective masks (surgical and FFP2) available in 2009 to **145 million surgical masks in 2020** (“We are not going to manage mask stocks, it is expensive, because we have to destroy them every five years. *Nous n’allons pas gérer des stocks de masques, c’est coûteux, parce qu’il faut les détruire tous les cinq ans.*”) ([Le Monde 200506](#)).

However, France, thanks to Italy, had an important advantage: time. It had several weeks to learn from the events in Lombardy. When, on the weekend of 21 March, virtually from one day to the next, patients were pouring into the hospitals of the **Greater Paris Region**, the number of available intensive care unit beds had already increased from 1,400 to 2,000 during the preceding week. Furthermore, two years before, in a simulation of a major terrorist attack, France had tested the use of a high-speed **TGV train**

for transporting casualties. At the height of the COVID epidemic, more than 500 patients were evacuated from epidemic hotspots like Alsace and the Greater Paris area to regions with fewer COVID-19 cases. Specially adapted high-speed trains as well as aircraft were employed, transporting patients as far away as Brittany and the Bordeaux area in the South-West, 600 km from Paris and 1000 km from Mulhouse. The French management of ICU beds was a huge logistical success.

Good virologists, huge lab network, family doctors (Germany)

Germany's fatality rate is lower than in other countries. It is assumed that the main reason for this difference is simply testing. While other countries were conducting a limited number of tests of older patients with severe cases of the virus, Germany was doing many more tests that included milder cases in younger people (Stafford 2020). The more people with no or mild symptoms you test, the lower the fatality rate. Reliable PCR methods had been developed by the end of January from the Drosten group at Berlin's Charité (Corman 2020).

Furthermore, in Germany's public health system, SARS-CoV-2 testing is not restricted to a central laboratory as in many other nations but can be conducted at quality-controlled laboratories throughout the country. Within a few weeks, overall capacity reached half a million PCR tests a week. The same low fatality rate is seen in South Korea, another country with high testing rates.

Finally, another important reason for the low mortality in Germany might be age distribution. During the first weeks of the epidemic, most people became infected during carnival sessions or ski holidays. The majority were younger than 50 years of age. Mortality in this age group is markedly lower than in older people.

Herd immunity (Sweden)

Sweden has never really imposed a lockdown, counting on the population to adopt individual social distancing and other protective measures to curb the transmission of SARS-CoV-2. As a result, Sweden has today (7 June 2020) a death rate of 461 per million population which compares unfavorably with Denmark (101) and Norway (44), with most deaths occurring in care homes and immigrant communities. Surprisingly, an [initial antibody survey in Stockholm](#) found that only about 7% of residents had been infected with SARS-COV-2 at the end of April. Still worse, Sweden didn't benefit economically of its no-lockdown approach as its economical performance seems to contract at a similar rate as countries in the rest of Europe ([Financial Times](#), 10 May 2020). For a detailed discussion of herd immunity, see [Randolph 2020](#).

Deferred beginning (South America)

In the initial months of 2020, the number of cases were comparatively low in South America ([Haider 2020](#)). As a matter of fact, the local epidemics took off roughly 4 weeks later than in Europe (see www.worldometers.info/coronavirus). Whether this delay is due only to a deferred import of SARS-CoV-2 from the initial outbreak region in China or to other factors (sunshine intensity? [Guasp 2020](#)) is unknown. However, according to WHO, South America has not become the [new epicenter of the coronavirus pandemic](#), with Brazil (374,000 cases and more than 23,000 deaths as of 27 May) reporting more cases than any other country in South America.

Splendid isolation (New Zealand, Australia)

With 102 deaths in Australia, 21 in New Zealand and no deaths in French Polynesia, Fiji, New Caledonia and Papua New Guinea, Oceania is the least hit area in the world. The geographical isola-

tion may allow these countries to become the first non-COVID zones in the world. International travel to New Zealand and Australia is still banned and may be subject to quarantine measures for quite a time.

The unknown outcome

The transmissibility of SARS-CoV-2, combined with the scarcity of crucial health equipment and facilities and the challenges of implementing widespread case isolation ([Wells 2020](#)), was supposed to have a devastating impact on African countries. Until now, these predictions have not come true. Although a few hundred deaths have been reported from a small number of countries (<10), the African COVID-19 epidemic is in no way comparable to the situation in Asia, Europe and the Americas.

However, caution should be used before hypothesizing an “African exception” due to factors such as demographics (huge young populations) or previous ‘exposure to more and different pathogens’. Some official figures may be underestimates, voluntary or not, due to regional difficulties in reporting. In some cities, such as [Kano](#), Nigeria, major outbreaks may already be under way. The [New York Times](#) reported on 17 May, “so many doctors and nurses have been infected with SARS-CoV-2 that few hospitals are now accepting patients”. Gravediggers would be working overtime. In Mogadishu, Somalia, officials say burials had tripled, according to the same report. In Tanzania, the US embassy has warned of the risk of “exponential growth” of COVID-19 cases in the country, adding that hospitals were “overwhelmed” ([The Guardian](#), 19 May).

It is too soon to say, how COVID-19 will evolve in Africa. As the situation in South America illustrates, on the scale of continents, the pandemic can “be late” by some weeks or months and still hit very hard.

Lockdown Exit

In the next months, all countries will have to find a balance between a maximum of economic activity and a still manageable number of patients in ICUs. Lockdown exit strategies should always include

- Strengthening of the national testing capacities to ensure access to PCR to all those in need;
- Effective contact-tracing system;
- Isolation capacities for positive people and close contacts.

Not all countries are able to fulfill these essential requirements, raising concerns about the possibility of new clusters and outbreaks. To facilitate identifying contacts at risk, several countries are considering developing smartphone applications that would record when other phones are coming into close contact and send an alert message in case one of these would have tested positive. However, [opinions are still divided](#) between centralized systems, where individual data would be stored in a central government server, and a decentralized system, where data will be stored in the mobile phone only. No common system has been agreed upon and the feasibility and usefulness of these apps still needs to be proven.

At the beginning of June 2020, most countries had started normalizing and restoring economic and societal activities. European borders will open again and tourism is expected to take off, albeit at a much reduced level (–50%?) compared to previous years.

Austria and **Germany** have eased lockdown measures for around 6 weeks, and apart from a few clusters in Germany, there is currently no indication of an imminent second “cataclysmic wave of contagion”, as the authors feared in previous editions. **Italy** started “Phase 2” on 4 May, with four million people re-

turning to their workplaces, shops opening and relaxed restriction on the movements of people, including visiting relatives. The number of new cases and deaths continues to decline everywhere except in Lombardy. However, schools will remain closed until September. **Spain** also slightly relaxed the lockdown measures on 2-4 May allowing greater movements and outdoor sport activities. **France** partially ended the lockdown on 11 May, with the country divided in a “red zone” where stricter restrictions will still apply, and “green zones” where they will be gradually relaxed.

In the **USA**, states have set their own timetables for imposing and easing lockdown measures, with a general trend in resuming activities despite the ongoing spread of the virus and high mortality from COVID-19. The government of the **United Kingdom**, the latecomer in European lockdown, announced the easing of the measures for 15 June.

Sweden has never really imposed a lockdown, counting on the population to adopt individually social distancing and other protective measures. Given the observed high death rate compared to neighbouring countries, there is now public pressure to implement stricter lockdown measures.

In all countries, most activities will eventually resume, but the tight-rope walk between maximising economic output and avoiding a new COVID-19 outbreak will need careful evaluation and considerations would include:

- Recommend frequent handwashing, disinfection, distancing (shops) and face masks (transport and other public places);
- Order distancing in cinemas, theaters and operas, and consider preventive contact tracing schemes in case an attendant should later be found to be infected;

- Ban events and activities that put people at less than one meter of distance (sports events, concerts, disco, festivals, pubs, etc.);
- Implement mandatory requirements of wearing face masks in public ([Anfinrud 2020](#));
- In the case of local outbreaks, recommend limitation in the movement of people and consider applying special restrictions to population groups at higher risk (e.g., elderly people, people with health conditions that put them at risk of severe COVID-19).

Some activities might remain closed for an unknown period of time, possibly until the availability of a vaccine.

In some countries, including the US, the epidemic is far from over with many new cases and deaths being reported every day. Therefore, it looks like the decision to exit the lockdown is more driven by economic necessity than justified by a satisfactory epidemiological situation. In this “second half” of the match “COVID vs. Humanity”, the economists are coming back and scoring more goals than the public health officials.

The economic impact of the COVID-19 pandemic is certainly unprecedented. The International Monetary Fund (IMF) forecasts a contraction of 3% of the planet’s GDP in 2020. In a recession like no other in peacetime for nearly a century, the countries of the Euro zone, the United States and the United Kingdom might see a contraction in activity of between 5.9% and 7.5%¹. Economical-

¹ The global CO₂ emissions decreased by 17% by early April 2020 compared with the mean 2019 levels, just under half from changes in surface transport (cars, truck, buses) ([Le Quéré 2020](#)). More than one billion tons of carbon emissions less. At their peak, emissions in individual countries decreased by an average of 26%, admittedly extreme and probably unseen before, but just

ly, socially, and politically, protracted lockdown is unsustainable. What can be done once – self-isolation of the population for months and months – can probably not be repeated.

“COVID Pass”

In countries with large COVID-19 outbreaks, tens of thousands of people died. Those who survive severe or less severe illness, with or without hospitalization, will have developed antibodies against the SARS-CoV-2 virus ([Zhang 2020](#), [Okba 2020](#)). Even more people, those who were infected but developed no symptoms, will have antibodies, too. Already, millions of people in China, Italy, Spain, France, and the US have developed SARS-CoV-2 antibodies.

In early June 2020, we still cannot be sure if and for how long these antibodies protect against a second infection. On 24 April, WHO issued a Scientific Brief stating that “There is no evidence yet that people who have had COVID-19 will not get a second infection” ([WHO 200424](#)). However, recently, neutralizing antibodies against SARS-CoV-2 were detected in virtually all hospital staff sampled from 13 days after the onset of COVID-19 symptoms (n=160) ([Fafi-Kremer 2020](#); see [Le Monde](#), 27 May) and there is no reason why they should not, since even symptomatic people recover from the infection, and most researchers think, based on our general knowledge of coronavirus infection, that

to the level of emissions in 2006. The impact on 2020 annual emissions will depend on the duration of the confinement, with a low estimate of -4% if pre-pandemic conditions return by mid-June, and a high estimate of -7% if some restrictions remain worldwide until the end of 2020. These figures are comparable to the rates of decrease needed year-on-year over the next decades to [limit climate change to a 1.5°C warming](#).

neutralizing antibodies are likely to be protective. Though further studies are needed to support this, it is very likely that once people have recovered from SARS-CoV-2 infection, they would not be vulnerable to a secondary infection and, even if they had a mild infection, they would be unlikely to infect others.

This has led to speculations about the possible introduction of a SARS-CoV-2 antibody passport, or a *COVID Pass*. People with neutralizing antibodies – assumed to be protected following a symptomatic or asymptomatic COVID-19 infection and therefore unable to transmit the virus – would be allowed to freely move around. Chile, Germany, and the UK, among others, considered implementing certifications that a person has contracted and recovered from COVID-19. These “licenses” would then allow immune people to engage in economic activity and provide safer care for vulnerable populations. The intention was to develop population-level ‘shield immunity’ by amplifying the proportion of interactions with recovered individuals relative to those of individuals of unknown status (Weitz 2020).

Major concerns remain as community licensing could stigmatize people, undermining the value of equal treatment. Immunity-based licenses would require therefore careful implementation to be ethical in practice (Persad 2020) and there are at least 10 good reasons why COVID (or immunity) passes are a bad idea (Kofler 2020), foremost because restricting liberty on the basis of biology threatens freedom, fairness and public health.

For the time being, a confirmed positive SARS-CoV-2 serological positivity might be useful in health care settings to determine who, among the health workers, should be allowed to work in close contact with confirmed or suspected COVID-19 patients.

The second wave

For now, in June 2020, the second wave of the COVID pandemic, as hypothesized in a study by Ferguson (Ferguson 2020; figure 7)

has not yet materialized. The study predicted that for as long as most people had no immunity against SARS-CoV-2, the lifting of strict “Stay at home” measures such as extreme social distancing and home quarantines would inevitably make the epidemic bounce back.

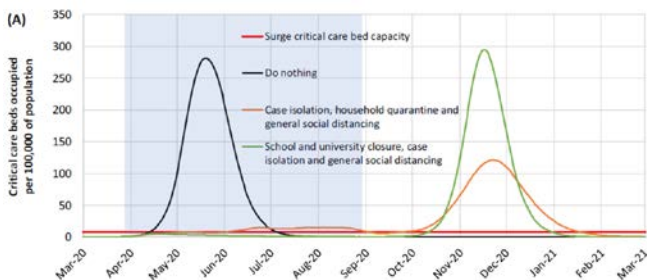


Figure 7. Impact of non-pharmaceutical interventions (NPIs) to reduce COVID-19 mortality and healthcare demand (Source: [Ferguson 2020](#)).

However, the world has changed. Today, a fever, a cough, anosmia and many other more subtle COVID symptoms will usually trigger an immediate cascade of action to prove or refute an acute SARS-CoV-2 infection. An existing acute infection, for its part, will trigger a similarly immediate cascade of contact tracing, testing, and quarantining. In addition, many people, while waiting for the coming episodes of the pandemic to unfold, have changed their behavior and avoid mass gatherings. They have understood that restrictive social-distancing measures will need to be combined with widespread testing and contact tracing to end the ongoing pandemic ([Giordano 2020](#) + less realistic, [Peto 2020](#)).

Herd immunity, the notion introduced to a wider public by a foolish politician, will not not be on the agenda for a long time. As for now, not a single country is anywhere close to reaching

herd immunity. Even in past hotspots like Wuhan, the prevalence of SARS-CoV-2 IgG positivity was 9.6% among 1,021 people applying for a permission (the SARS-CoV-2 nucleic acid test needed to be negative) (Wu X 2020). A French study projected 2.8 million or 4.4% (range: 2.8–7.2) prevalence of infections in France. In Los Angeles, the prevalence of antibodies was 4.65% (Sood 2020). (And even this low number may be biased because symptomatic persons may have been more likely to participate.) A recent nationwide coronavirus antibody study In Spain showed that about 5% of the population had contracted the virus. These infection rates are clearly insufficient to avoid a second wave of a SARS-CoV-2 epidemic (Salje 2020).

Coronaviruses have come a long way (Weiss 2020) and will stay with us for a long time. Questions abound: When will we move freely around the world as we did before? How many years will air traffic need to return to pre-COVID-19 levels? Will we be inclined to plan vacations nearer to home than at the other side of the globe? Will we wear face masks for years? Will there be any nightlife event with densely packed people dancing and shouting and drinking in any city of the world anytime soon?

The French have an exquisitely precise formula to express unwillingness for living in a world you do not recognize: “Un monde de con!” Fortunately, we will be able to walk out of this *monde de con* thanks to a scientific community which is larger, stronger, and faster than at any time in history. (BTW, should politicians who are skeptical of science be ousted out of office? Yes, please! It is about time now!) As of today, we do not know how long lasting, how intense, and how deadly this pandemic will be. We are walking on moving ground and, in the coming months, we will need to be flexible, resilient, and inventive, looking for and finding solutions nobody would have imagined just months ago. Sure enough, though, science will lead the way

out. If we could leap three years into the future and read the story of COVID-19, we would not believe our eyes.

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2. Transmission

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Viruses have substantially influenced human health, interactions with the ecosphere, and societal history and structures (Chappell 2019). In a highly connected world, microbial evolution is boosted and pathogens exploit human behaviors to their own benefit (Morens 2013). This was critically shown during the SARS epidemic in 2003 (Kamps-Hoffmann 2003), the outbreak of Middle East Respiratory Syndrome coronavirus (MERS-CoV) (Zaki 2012), the last great Ebola epidemic in West Africa (Arwady 2015, Heymann 2015) and the Zika epidemic in 2015-2017 (Fauci 2016). Over the same time period, more virulent strains of known respiratory pathogens – H5N1 influenza virus, tuberculosis, avian H7N9 influenza virus – have emerged (Kamps-Hoffmann 2006, Jassal 2009, Gao 2013).

The Virus

SARS-CoV-2, Severe Acute Respiratory Syndrome coronavirus 2, is a highly transmissible ‘complex killer’ (Cyranoski 2020) that forced half of humanity, 4 billion people, to bunker down in their homes in the early spring of 2020. The respiratory disease rapidly evolved into a pandemic (Google 2020). In most cases, the illness is asymptomatic or paucisymptomatic and self-limited. A subset of infected individuals has severe symptoms and sometimes prolonged courses (Garner 2020). Around 10% of infected people need hospitalization and around one third of them treatment in intensive care units. The overall mortality rate of SARS-CoV-2 infection seems to be less than 1%.

Coronaviruses are tiny spheres of about 70 to 80 nanometers (a millionth of a millimeter) on thin-section electron microscopy ([Perlman 2019](#)). Compared to the size of a human, SARS-CoV-2 is as small as a big chicken compared to the planet Earth ([El País](#)). The *raison d'être* of SARS-CoV-2 is to proliferate, like that of other species, for example *H. sapiens sapiens* who has been successful in populating almost every corner of the world, sometimes at the expense of other species. SARS-CoV-2, for now, seems to be on a similarly successful track. By 7 June, only a handful of countries can claim to have been spared by the pandemic.

SARS-CoV-2's global success has multiple reasons. The new coronavirus hijacks the human respiratory system to pass from one individual to another when people sneeze, cough, shout and speak. It is at ease both in cold and in warm climates; and, most importantly and unlike the two other deadly coronaviruses SARS-CoV and MERS-CoV, it manages to get transmitted to the next individual before it develops symptoms in the first one (see below, Asymptomatic Infection, page 83). There is no doubt that SARS-CoV-2 has a bright future – at least until the scientific community develops a safe and efficient vaccine (see the chapter *Immunology*, page 125).

SARS-CoV-2 and its kin

SARS-CoV-2 is a **coronavirus** like

- SARS-CoV (its cousin of the 2002/2003 epidemic),
- MERS-CoV (Middle East Respiratory Syndrome coronavirus),
- and a group of so-called CAR coronaviruses (for Community-Acquired Respiratory CoVs: 229E, OC43, NL63, HKU1) which account for 15 to 30% of common colds.

The CAR group viruses are highly transmissible and produce about 15 to 30% of the common colds, typically in the winter

months. On the contrary, SARS-CoV and MERS-CoV have case fatality rates of 10% and 34%, respectively, but they never achieved pandemic spread. SARS-CoV-2, from a strictly viral point of view, is the shooting star in the coronavirus family: it combines high transmissibility with high morbidity and mortality.

SARS-CoV-2 is a **virus** like other commonly known viruses that cause human disease such as hepatitis C, hepatitis B, Ebola, influenza and human immunodeficiency viruses. (Note that the differences between them are bigger than between humans and amebas.) With the exception of influenza, these viruses have a harder time infecting humans than SARS-CoV-2. **Hepatitis C virus (HCV)**, a major cause of chronic and often fatal liver disease, is mainly transmitted by percutaneous exposure to blood, by unsafe medical practices and, less frequently, sexually. The **human immunodeficiency virus (HIV)**, in addition to exposure to blood and perinatal transmission, also exploits sexual contact as a potent transmission route. **Hepatitis B virus (HBV)** is an even more versatile spreader than HCV and HIV as it can be found in high titers in blood, cervical secretions, semen, saliva, and tears; even tiny amounts of blood or contaminated secretions can transmit the virus. Ideal infection environments for HBV include, for example, schools, institutions and hospitals where individuals are in close and prolonged contact.

Of note, apart from HIV and hepatitis B and C, most viral diseases have no treatment. For example, there is no treatment for measles, polio, or smallpox. For influenza, decades of research have produced two specific drugs which have not been able to demonstrate to reduce mortality – despite tests on thousands of patients. After 35 years of research, there is still no vaccine to prevent HIV infection.

Ecology of SARS-CoV-2

SARS-CoV-2 is present at high concentrations in the upper and lower respiratory tract (Zhu N 2020, Wang 2020, Huang 2020). The virus has also been found, albeit at low levels, in the kidney, liver, heart, brain, and blood (Puelles 2020). Outside the human body, the virus has been shown to be detectable as an aerosol (in the air) for up to three hours, up to 24 hours on cardboard and up to two to three days on plastic and stainless steel (van Doremalen 2020). Another study documented contamination of toilets (toilet bowl, sink, and door handle) and air outlet fans (Ong SWX 2020). This is in line with the experience from MERS where many environmental surfaces of patients' rooms, including points frequently touched by patients or healthcare workers, were contaminated by MERS-CoV (Bin 2016).

Person-to-Person Transmission

Person-to-person transmission of SARS-CoV-2 was established within weeks of identification of the first cases (Chan JF 2020, Rothe 2020). Shortly after, it was suggested that asymptomatic individuals would probably account for a substantial proportion of all SARS-CoV-2 transmissions (Nishiura 2020, Li 2020). Viral load can be high 2-3 days before the onset of symptoms and almost half of all secondary infections are supposed to be caused by presymptomatic patients (He 2020).

A key factor in the transmissibility of SARS-CoV-2 is the high level of virus shedding in the upper respiratory tract (Wolfel 2020), even among paucisymptomatic patients. Pharyngeal virus shedding is very high during the first week of symptoms, with a peak at $>7 \times 10^8$ RNA copies per throat swab on day 4. Infectious virus was readily isolated from samples derived from the throat or lung. That distinguishes it from SARS-CoV, where replication occurred mainly in the lower respiratory tract (Gandhi 2020);

SARS-CoV and MERS-CoV infect intrapulmonary epithelial cells more than cells of the upper airways (Cheng PK 2004, Hui 2018).

The shedding of viral RNA from sputum appears to outlast the end of symptoms and seroconversion is not always followed by a rapid decline in viral load (Wolfel 2020). This contrasts with influenza where persons with asymptomatic disease generally have lower quantitative viral loads in secretions from the upper respiratory tract than from the lower respiratory tract and a shorter duration of viral shedding than persons with symptoms (Ip 2017).

Routes of Transmission

Respiratory droplets vs aerosol

SARS-CoV-2 is spread predominantly via virus-containing droplets through sneezing, coughing, or when people interact with each other for some time in close proximity (usually less than one metre) (ECDC 2020, Chan JF 2020, Li Q 2020, Liu Y 2020). These droplets can then be inhaled or land on surfaces where they can be detectable for up to four hours on copper, up to 24 hours on cardboard and up to two to three days on plastic and stainless steel (van Doremalen 2020). Other people may come into contact with these droplets and get infected when they touch their nose, mouth or eyes.

SARS-CoV-2 was thought to be transmitted primarily through larger droplet particles, $>5\text{--}10\text{ }\mu\text{m}$ in diameter, commonly referred to as **respiratory droplets**, which fall to the ground attracted by gravity. In the beginning of the pandemic SARS-CoV-2 was NOT thought to be transmitted via smaller particles, $<5\mu\text{m}$ in diameter, which are referred to as droplet nuclei or **aerosol**. Recently, however, some authors have voiced concern that SARS-CoV-2 could also be spread via aerosol. They point to episodes during the 2003 SARS epidemic when an airborne route

of transmission appeared to be a plausible explanation for the so-called [Amoy Garden outbreak](#). On that occasion, the virus was aerosolized within the confines of very small bathrooms and may have been inhaled, ingested or transmitted indirectly by contact with fomites as the aerosol settled ([WHO 2003](#)). Other authors suggest that ‘Heating, Ventilation and Air Conditioning Systems’ (HVAC) when not adequately used may contribute to the transmission of the virus, as suggested by descriptions from Japan, Germany, and the Diamond Princess Cruise Ship ([Correia 2020](#), [Gormley 2020](#)). As a matter of fact, SARS-CoV-2 has been shown to be detectable as an aerosol (in the air) for up to three hours ([van Doremalen 2020](#)) and in patients’ toilet areas ([Liu Y 2020](#)).

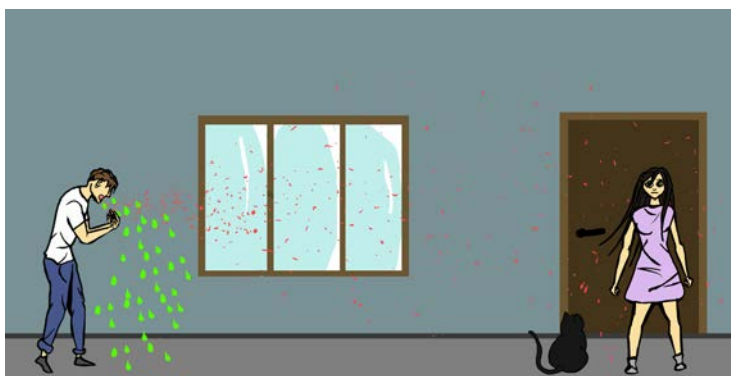


Figure 1. Transmission of a respiratory virus. 1) After coughing, sneezing, shouting and even after speaking – particularly loud speaking–, large droplets (green) drop to the ground around the young man. 2) In addition, some droplets, small and lightweight enough (red), are transported by air currents over longer distances. Whether the second – aerosol – transmission is an epidemiologically relevant transmission route in the SARS-CoV-2 pandemic, is currently being discussed. Adapted from [Morawska 2020](#). Art work: [Félix Prudhomme](#); YouTube: [IYENSS](#). (This and the following illustration are under free license if credited correctly.)

Experimental support for these concerns comes from studies that visualize droplet formation at the exit of the mouth during violent expiratory events such as sneezing and coughing (Scharfman 2016, Bourouiba 2020; see also the [video](#)). These studies show that the lifetime of a droplet can be considerably longer than previously assumed. When analyzed with highly sensitive laser light scattering, loud speech was found to be able to emit thousands of oral fluid droplets per second which could linger in the air for minutes (Anfinrud 2020, Stadnytskyi 2020; see also the [movies](#) showing the experimental setup). Loud and persistent shouting as would be usual in noisy, closed and stagnant air environments (meat-packing facilities, discos, pubs, etc.) is now believed to produce the same number of droplets as produced by coughing (Chao 2020). Speech and other vocal activities such as singing have also been shown to generate air particles, with the rate of emission corresponding to voice loudness (Asadi 2019). Confined public spaces (e.g., restrooms or elevators) were discussed as a favorable environment in an outbreak in Wenzhou, China (Cai J 2020). Of note, several outbreaks are now linked to choir practices in the Netherlands, Germany and the US (Hamner 2020) (see also the chapter Epidemiology, page 19).

The question of whether SARS-CoV-2 is transmitted only via respiratory droplets (see a recent transmission experiment among hACE2 mice; Bao L 2020) or also via aerosol is crucial for the implementing of future prevention measures. In the former case, the current prevention recommendations of frequent hand-washing and maintaining a distance of at least one meter (arm's length) (WHO 2020a) could be sufficient. In the case of proven airborne transmission over several meters, however, current distancing measures would need to be adapted, with far-reaching implications for cultural and economic life (theaters,

cinemas, restaurants, pubs, shops, etc.). Some authors plead that the international and national authorities acknowledge the reality that the virus spreads through air, and recommend that adequate control measures be implemented to prevent further spread of the SARS-CoV-2 virus (Morawska 2020), including wearing suitable masks whenever infected persons may be nearby and providing adequate ventilation of enclosed spaces (Somsen 2020) where such persons are known to be or may recently have been (Meselson 2020).

The current evidence for aerosol transmission and resulting recommendations for prevention have been sublimely summarized by Prather et al. in five sentences: “Respiratory infections occur through the transmission of virus-containing droplets (>5 to $10\ \mu\text{m}$) and aerosols ($\leq 5\ \mu\text{m}$) exhaled from infected individuals during breathing, speaking, coughing, and sneezing. Traditional respiratory disease control measures are designed to reduce transmission by droplets produced in the sneezes and coughs of infected individuals. However, a large proportion of the spread of coronavirus disease 2019 (COVID-19) appears to be occurring through airborne transmission of aerosols produced by asymptomatic individuals during breathing and speaking (Morawska 2020, Anderson 2020, Asadi 2019). Aerosols can accumulate, remain infectious in indoor air for hours, and be easily inhaled deep into the lungs. For society to resume, measures designed to reduce aerosol transmission must be implemented, including universal masking and regular, widespread testing to identify and isolate infected asymptomatic individuals (Prather 2020).”

Fomites

It is currently unclear whether and to which extent transmission of via fomites (e.g., elevator buttons, hand rails, restroom taps) is epidemiologically relevant (Cai J 2020). (A fomite is any inanimate object that, when contaminated with or exposed to infec-

tious agents such as a virus, can transfer a disease to another person).

Mother-to-child

Mother-to-child transmission doesn't seem to be a prominent route of SARS-CoV-2 transmission. There is one report of a newborn with elevated SARS-CoV-2 IgM antibodies who was exposed for 23 days from the time of the mother's diagnosis of COVID-19 to delivery ([Dong L 2020](#)). However, there was no evidence for intrauterine vertical transmission among another group of nine women with COVID-19 pneumonia in late pregnancy ([Chen H 2020](#)).

Vaginal (n=24) versus elective cesarean (n=16) was addressed in a study from Northern Italy. In one case a newborn had a positive test after a vaginal operative delivery.

Two women with COVID-19 breastfed without a mask because infection was diagnosed in the post-partum period; their newborns tested positive for SARS-CoV-2 infection. The authors conclude that although post-partum infection cannot be excluded with 100% certainty, vaginal delivery seems to be associated with a low risk of intrapartum SARS-CoV-2 transmission ([Ferrazzi 2020](#)).

In at least two cases, SARS-CoV-2 has been found in breast milk ([Wu Y 2020](#), [Groß 2020](#)). As of May 2020, the Italian Society on Neonatology (SIN), endorsed by the Union of European Neonatal & Perinatal Societies (UENPS), recommended breastfeeding as advisable if a mother previously identified as COVID-19-positive or under investigation for COVID-19 was asymptomatic or paucisymptomatic at delivery. On the contrary, when a mother with COVID-19 is too sick to care for the newborn, the neonate should be managed separately and fed freshly expressed breast milk ([Davanzo 2020](#), [Davanzo 2020b \[Italian\]](#)). This guidance may be subject to change in the coming months.

Stool, urine

Although no cases of fecal-oral transmission of SARS-CoV-2 have been reported thus far, a study from Zhuhai reports prolonged presence of SARS-CoV-2 viral RNA in fecal samples. Of the 41 (55%) of 74 patients with fecal samples that were positive for SARS-CoV-2 RNA, respiratory samples remained positive for SARS-CoV-2 RNA for a mean of 17 days and fecal samples remained positive for a mean of 28 days after first symptom onset (Wu Y 2020). In 22/133 patients, SARS-CoV-2 was still detected in the sputum or feces (up to 39 and 13 days, respectively) after pharyngeal swabs became negative (Chen 2020).

Until proof of the contrary, the possibility of fecal-oral transmission should not be excluded. Strict precautions must be observed when handling the stools of patients infected with coronavirus. Sewage from hospitals should also be properly disinfected (Yeo 2020). Fortunately, antiseptics and disinfectants such as ethanol or bleach have good activity on human coronaviruses (Geller 2012). During the SARS-CoV outbreak in 2003, where SARS-CoV was shown to survive in sewage for 14 days at 4°C and for 2 days at 20°C (Wang XW 2005), environmental conditions could have facilitated this route of transmission.

Blood products

SARS-CoV-2 is rarely detected in blood (Wang W 2020, Wolfel 2020). After screening of 2,430 donations in real-time (1,656 platelet and 774 whole blood), authors from Wuhan found plasma samples positive for viral RNA from 4 asymptomatic donors (Chang 2020). It remains unclear whether detectable RNA signifies infectivity.

In a Korean study, seven asymptomatic blood donors were later identified as COVID-19 cases. None of 9 recipients of platelets or red blood cell transfusions tested positive for SARS-CoV-2 RNA

(Kwon 2020). More data are needed before transmission through transfusion can be declared safe.

Sexual transmission

It is unknown whether purely sexual transmission is possible. Scrupulously eluding infection via fomites and respiratory droplets during sexual intercourse would suppose remarkable acrobatics many people might not be willing to perform.

Cats and dogs

SARS-CoV-2 can be transmitted to cats and dogs. When inoculated with SARS-CoV-2, three cats transmitted the virus to three other cats. None of the cats showed symptoms, but all shedded virus for 4 to 5 days and developed antibody titers by day 24 (Halfmann 2020). In another study, two out of fifteen dogs from households with confirmed human cases of COVID-19 in Hong Kong were found to be infected. The genetic sequences of viruses from the two dogs were identical to the virus detected in the respective human cases (Sit 2020). It is too early to know if cats and dogs are potential intermediate hosts in chains of human-pet-human transmission.

Transmission Event

Transmission of a virus from one person to another depends on four variables:

1. The nature of the **virus**;
2. The nature of the **transmitter**;
3. The nature of the **transmittee** (the person who will become infected);
4. The transmission **setting**.

Virus

In order to stay in the evolutionary game, all viruses have to overcome a series of challenges. They must attach to cells; fuse with their membranes; release their nucleic acid into the cell; manage to make copies of themselves; and have the copies exit the cell to infect other cells. In addition, respiratory viruses must make their host cough and sneeze to get back into the environment again. Ideally, this happens before the hosts realize that they are sick. This is all the more amazing as SARS-CoV-2 is more like a piece of computer code than a living creature *in sensu strictu* (its 30,000 DNA base pairs are a mere 100,000th of the human genetic code). That doesn't prevent the virus from being ferociously successful:

- It attaches to the human angiotensin converting enzyme 2 (ACE2) receptor (Zhou 2020) which is present not only in nasopharyngeal and oropharyngeal mucosa, but also in lung cells, such as in type II pneumocytes. SARS-CoV-2 thus combines the high transmission rates of the common coronavirus NL63 (infection of the upper respiratory tract) with the severity of SARS in 2003 (lower respiratory tract);
- It has a relatively long incubation time of around 5 days (influenza: 1-2 days), thus giving it more time to spread;
- It is transmitted by asymptomatic individuals.

As mentioned above, SARS-CoV-2 can be viable for days (van Doremalen 2020). Environmental factors that might influence survival of the virus outside the human body will be discussed below (page 87).

The virologic determinants of more or less successful SARS-CoV-2 transmission are not yet fully understood.

Transmittor

Infectiousness seems to peak on or before symptom onset (He X 2020), with around half of secondary cases being possibly infected during the presymptomatic stage. The mean incubation is around 5 days (Lauer 2020, Li 2020, Zhang J 2020, Pung 2020), comparable to that of the coronaviruses causing SARS or MERS (Virlogeux 2016). Almost all symptomatic individuals will develop symptoms within 14 days of infection, beyond that only in rare cases (Bai Y 2020).

It is currently unknown if SARS-CoV-2 transmission correlates with the following characteristics of the index case (transmitter):

- Symptom severity;
- Large concentrations of virus in the upper and lower respiratory tract;
- SARS-CoV-2 RNA in plasma;
- In the future: reduced viral load due to drug treatment (as in people treated for HIV infection) [Cohen 2011, Cohen 2016, LeMessurier 2018])

SARS-CoV-2 transmission certainly correlates with a still ill-defined “super-spreader status” of the infected individual. For unknown reasons, some individuals – so-called super-spreaders – are remarkably contagious, capable of infecting dozens or hundreds of people, possibly because they breathe out many more particles than others when they talk (Asadi 2019), shout, cough or sneeze.

Transmission is more likely when the infected individual has few or no symptoms. **Asymptomatic transmission** of SARS-CoV-2 – proven a few weeks after the beginning of the pandemic (Bai Y 2020) – has justly been called the Achilles’ heel of the COVID-19 pandemic (Gandhi 2020). As shown during an outbreak in a

skilled nursing facility, the percentage of asymptomatic individuals can be as high as 50% early (Arons 2020); note that most of these individuals would later develop some symptoms. Importantly, SARS-CoV-2 viral load was comparable in individuals with typical and atypical symptoms, and in those who were pre-symptomatic or asymptomatic. Seventeen of 24 specimens (71%) from presymptomatic persons had viable virus by culture 1 to 6 days before the development of symptoms (Arons 2020), suggesting that SARS-CoV-2 may be shed at high concentrations before symptom development. It is assumed that about 50% of all infections occur through presymptomatic transmission (He X 2020).

To what extent **children** contribute to the spread of SARS-CoV-2 infection in a community is unknown. Infants and young children are normally at high risk for respiratory tract infections. The immaturity of the infant immune system may alter the outcome of viral infection and is thought to contribute to the severe episodes of influenza or respiratory syncytial virus infection in this age group (Tregoning 2010). Until now, however, there is a surprising absence of pediatric patients with COVID-19, something that has perplexed clinicians, epidemiologists, and scientists (Kelvin 2020). Although the discovery of a pediatric inflammatory multisystem syndrome (PIMS) in SARS-CoV-2 infection in children (Verdoni 2020, Viner 2020, ECDC 15 May 2020) came as a surprise, the fact that children are susceptible to SARS-CoV-2 infection but frequently do not have notable disease raises the possibility that children could be an important source of viral transmission and amplification in the community. There is an urgent need for further investigation of the role children have in SARS-CoV-2 transmission chains (Kelvin 2020).

SARS-CoV-2 is highly transmissible, but given the right circumstances and the right prevention precautions, **zero transmission** can be achieved. In one case report, there was no evidence of transmission to 16 close contacts, among them 10 high-risk

contacts, from a patient with mild illness and positive tests for up to 18 days after diagnosis ([Scott 2020](#)).

Transmittee

Upon exposure to SARS-CoV-2, the virus may come in contact with cells of the upper or lower respiratory tract of an individual. Numerous cell entry mechanisms of SARS-CoV-2 have been identified that potentially contribute to the immune evasion, cell infectivity, and wide spread of SARS-CoV-2 ([Shang J 2020](#)). (The pathogenesis of COVID-19 will be discussed in an upcoming separate COVID Reference chapter.) Susceptibility to SARS-CoV-2 infection is probably influenced by the host genotype ([Williams 2020](#)). This would explain the higher percentage of severe COVID-19 in men ([Piccininni 2020](#)) and possibly the similar disease course in some twins in the UK ([The Guardian](#), 5 May 2020).

A high percentage of SARS-CoV-2 seronegative individuals have SARS-CoV-2 reactive T cells. This is explained by previous exposure to other coronaviruses (“common cold” coronaviruses) which have proteins that are highly similar to those of SARS-CoV-2. It is still unclear whether these cross-reactive T cells confer some degree of protection, are inconsequential or even potentially harmful if someone who possesses these cells becomes infected with SARS-CoV-2 ([Braun 2020](#), [Grifoni 2020](#)).

The “right” genotype may not be sufficient in the presence of massive exposure, for example by numerous infected people and on multiple occasions as might happen, for example, in health care institutions being overwhelmed during the beginning of an epidemic. It is known from other infectious diseases that viral load can influence the incidence and severity of disease. Although the evidence is limited, high infection rates among health workers have been attributed to more frequent contact with infected patients, and frequent exposure to excretia with high viral load ([Little 2020](#)).

Transmission setting

The transmission setting, i.e., the actual place where the transmission of SARS-CoV-2 occurs, is the final element in the succession of events that lead to the infection of an individual. High population density which facilitates super-spreading events (see also chapter Epidemiology, *Transmission Hotspots*, page 20) are key to widespread transmission of SARS-CoV-2.

Super-spreading events

Transmission of SARS-CoV and MERS-CoV, too, occurred to a large extent by means of super-spreading events (Peiris 2004, Hui 2018). Super-spreading has been recognized for years to be a normal feature of disease spread (Lloyd-Smith 2005). One group suggested that 80% of secondary transmissions could be caused by a small fraction of infectious individuals (around 10%). A value called the dispersion factor (k) describes this phenomenon. The lower the k is, the more transmission comes from a small number of people (Kupferschmidt 2020). While SARS was estimated to have a k of 0.16 (Lloyd-Smith 2005) and MERS of 0.25, in the flu pandemic of 1918, in contrast, the value was about one, indicating that clusters played less of a role (Endo 2020). For the SARS-CoV-2 pandemic, the dispersion factor (k) is currently thought to be higher than for SARS and lower than for influenza (Endo 2020, Miller 2020, On Kwok 2020).

Examples of SARS-CoV-2 clusters have been linked to a wide range of mostly indoor settings (Leclerc 2020). In 318 clusters of three or more cases involving 1245 confirmed cases, only a single outbreak originated in an outdoor environment (Qian H 2020). In one study, the odds that a primary case transmitted COVID-19 in a closed environment was around 20 times greater compared to an open-air environment (Nishiura 2020).

Transmission clusters, partly linked to super-spreader events, have been reported since the very beginning of the SARS-CoV-2 pandemic:

- Business meeting, Southern Germany, 20-21 January ([Rothe 2020](#))
- Cruise Ship, Yokohama, Japan, 4 February ([Rocklov 2020](#))
- Church meeting, Daegu, Korea, 9 and 16 February ([Kim 2020](#))
- Religious gathering, Mulhouse, France, 17-24 February ([Kuteifan 2020](#))
- Advisory board meeting, Munich, Germany, 20-21 ([Hijnen 2020](#))
- Nursing facility, King County, Washington, 28 February ([McMichael 2020](#))
- Aircraft carriers: Theodor Roosevelt ([The Guardian](#)) + Charles-de-Gaulle, March ([Le Monde](#))
- Choir ([Hamner 2020](#))
- Homeless shelter, Boston, 28 March ([Baggett 2020](#))

Temperature and climate

Another variable still poorly understood is ambient temperature and humidity.

2003: SARS-CoV

The transmission of coronaviruses can be affected by several factors, including the climate ([Hemmes 1962](#)). Looking back to the 2003 SARS epidemic, we find that the stability of the first SARS virus, SARS-CoV, depended on temperature and relative humidity. A study from Hong Kong, Guangzhou, Beijing, and Taiwan suggested that the SARS outbreak in 2002/2003 was significantly associated with environmental temperature. The study

provided some evidence that there was a higher possibility for SARS to reoccur in spring than in autumn and winter (Tan 2005). It was shown that SARS-CoV remained viable for more than 5 days at temperatures of 22–25°C and relative humidity of 40–50%, that is, typical air-conditioned environments (Chan KH 2011). However, viability decreased after 24 h at 38°C and 80–90% relative humidity. The better stability of SARS coronavirus in an environment of low temperature and low humidity could have facilitated its transmission in subtropical areas (such as Hong Kong) during the spring and in air-conditioned environments. It might also explain why some Asian countries in the tropics (such as Malaysia, Indonesia or Thailand) with high temperature and high relative humidity environment did not have major community SARS outbreaks (Chan KH 2011).

2020: SARS-CoV-2

It is as yet unclear as to whether and to what extent climatic factors influence virus survival outside the human body and might influence local epidemics. SARS-CoV-2 is not readily inactivated at room temperature and by drying like other viruses, for example herpes simplex virus. One study mentioned above showed that SARS-CoV-2 can be detectable as an aerosol (in the air) for up to three hours, up to four hours on copper, up to 24 hours on cardboard and up to two to three days on plastic and stainless steel (van Doremalen 2020).

A few studies suggest that low temperature might enhance the transmissibility of SARS-CoV-2 (Triplett 2020; Wang 2020b, Tobías 2020) and that the arrival of summer in the northern hemisphere could reduce the transmission of the COVID-19. A possible association of the incidence of COVID-19 and both reduced solar irradiance and increased population density has been discussed (Guasp 2020). It was reported that simulated sunlight rapidly inactivated SARS-CoV-2 suspended in either simulated saliva

or culture media and dried on stainless steel plates while no significant decay was observed in darkness over 60 minutes (Ratnesar-Shumate 2020). However, another study concluded that transmission was likely to remain high even at warmer temperatures (Sehra 2020). In particular the current epidemics in Brazil and India – countries with high temperatures – should temper hopes that COVID “simply disappears like a miracle”. Warm and humid summer conditions alone might be unlikely to limit substantially new important outbreaks (Luo 2020, Baker 2020, Collins 2020).

Outlook

Less than 6 months after the first SARS-CoV-2 outbreak in China, the transmission dynamics driving the pandemic are coming into focus.

It now appears that a high percentage (as high as 80%?) of secondary transmissions could be caused by a small fraction of infectious individuals (as low as 10%?; Endo 2020); if this is the case, then the more people are grouped together, the higher the probability that a superspreader is part of the group.

It also appears that aerosol transmission might play an important role in SARS-CoV-2 transmission (Prather 2020); if this is the case, then building a wall around this same group of people and putting a ceiling above them further enhances the probability of SARS-CoV-2 infection.

It finally appears that shouting and speaking loudly emits thousands of oral fluid droplets per second which could linger in the air for minutes (Anfinrud 2020, Stadnytskyi 2020, Chao 2020, Asadi 2019); if this is the case, then creating noise (machines, music) around people grouped in a closed environment would create the perfect setting for a superspreader event.

Over the coming months, the scientific community will try and

- define more precisely the role of aerosols in the transmission of SARS-CoV-2;
- unravel the secrets of super-spreading;
- advance our understanding of host factors involved in the successful “seeding” of SARS-CoV-2 infection;
- elucidate the role of children in the transmission of the virus at the community level;
- continue to describe the conditions under which people should be allowed to gather in larger groups;

Without a coronavirus vaccine, nobody will return to a “normal” pre-2020 way of life. The most promising exit strategy for the coronavirus crisis is an efficient vaccine that can be rolled out safely and affordably to billions of people. Thousands of researchers are working around the clock, motivated by fame (becoming the next [Dr. Salk](#)?) and money (becoming the next [Scrooge McDuck](#)?). However, despite these efforts, it is not even certain that developing a COVID-19 vaccine is possible (Piot 2020, cited by [Draulens](#)). Until the worldwide availability of a vaccine, the only feasible prevention scheme is a potpourri of physical distancing ([Kissler 2020](#)), intensive testing, case isolation, contact tracing, quarantine ([Ferretti 2020](#)) and, as a last (but not impossible) resort, local lockdowns.

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- WHO 2020. **Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations**. 29 March 2020. Web page: <https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations> (accessed 15 May).
- Williams FMK, Freydin M, Mangino M, et al. **Self-reported symptoms of covid-19 including symptoms most predictive of SARS-CoV-2 infection, are heritable**. medRxiv, 27 April. Abstract: <https://www.medrxiv.org/content/10.1101/2020.04.22.20072124v2>
- Wilson NM, Norton A, Young FP, Collins DW. **Airborne transmission of severe acute respiratory syndrome coronavirus-2 to healthcare workers: a narrative review**. Anaesthesia. 2020 Apr 20. PubMed: <https://pubmed.gov/32311771>. Full-text: <https://doi.org/10.1111/anae.15093>
- Wolfel R, Corman VM, Guggemos W, et al. **Virological assessment of hospitalized patients with COVID-2019**. Nature. 2020 Apr 1. PubMed: <https://pubmed.gov/32235945>. Full-text: <https://doi.org/10.1038/s41586-020-2196-x>
- Wu J, Huang Y, Tu C, et al. **Household Transmission of SARS-CoV-2, Zhuhai, China, 2020**. Clin Infect Dis. 2020 May 11. pii: 5835845. PubMed: <https://pubmed.gov/32392331>. Full-text: <https://doi.org/10.1093/cid/ciaa557>
- Wu Y, Guo C, Tang L, et al. **Prolonged presence of SARS-CoV-2 viral RNA in faecal samples**. Lancet Gastroenterol Hepatol. 2020 Mar 19. pii: S2468-

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- Zhang J, Litvinova M, Wang W, et al. **Evolving epidemiology and transmission dynamics of coronavirus disease 2019 outside Hubei province, China: a descriptive and modelling study.** Lancet Infect Dis. 2020 Apr 2. pii: S1473-3099(20)30230-9. PubMed: <https://pubmed.gov/32247326>. Full-text: [https://doi.org/10.1016/S1473-3099\(20\)30230-9](https://doi.org/10.1016/S1473-3099(20)30230-9)
- Zhang Y, Li Y, Wang L, Li M, Zhou X. **Evaluating Transmission Heterogeneity and Super-Spreading Event of COVID-19 in a Metropolis of China.** Int J Environ Res Public Health. 2020 May 24;17(10):E3705. PubMed: <https://pubmed.gov/32456346>. Full-text: <https://doi.org/10.3390/ijerph17103705>.
- Zhu N, Zhang D, Wang W, et al. **A Novel Coronavirus from Patients with Pneumonia in China, 2019.** N Engl J Med 2020; 382:727-733. PubMed: <https://pubmed.gov/31978945> Full-text: <https://doi.org/10.1056/NEJMoa2001017>

3. Virology

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Coronaviruses are found in a variety of animals and humans. These enveloped viruses contain a single strand of positive-sense RNA. Virions are mostly spherical, with pronounced spiked glycoprotein (S) embedded in the envelope. Additional structural proteins include envelope (E), matrix (M), and nucleocapsid (N).

The family *Coronaviridae* includes four genera, alpha-, beta-, delta- and gammacoronavirus, as well as several subgenera and species. Phylogenetic analysis on the coronavirus genomes has revealed that SARS-CoV-2 is a new member of the betacoronavirus genus, which includes severe acute respiratory syndrome-related coronavirus (SARS-CoV), Middle East respiratory syndrome-related coronavirus (MERS-CoV), bat SARS-related coronaviruses (SARSr-CoV), as well as others identified in humans and diverse animal species. Intra- and inter-species transmission of CoVs, and genetic recombination events contribute to the emergence of new CoV strains.

SARS-CoV-2 is taxonomically related to the subgenus *Sarbecovirus* together with SARS-CoV and bat SARS-like CoVs. Genomic sequencing showed SARS-CoV-2 to be closely related to betacoronaviruses detected in bats, but distinct from SARS-CoV. The following sections includes some key papers on different topics. Please check the comments on these studies.

Taxonomy

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. **The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2.** Nat Microbiol. 2020 Apr;5(4):536-544. PubMed: <https://pubmed.gov/32123347>. Full-text: <https://doi.org/10.1038/s41564-020-0695-z>

A consensus statement defining the place of SARS-CoV-2 (provisionally named 2019-nCoV) within the Coronaviridae family.

Ceraolo C, Giorgi FM. **Genomic variance of the 2019-nCoV coronavirus.** J Med Virol. 2020 May;92(5):522-528. PubMed: <https://pubmed.gov/32027036>. Full-text: <https://doi.org/10.1002/jmv.25700>

Analysis of 56 genomic sequences from distinct patients, showing high sequence similarity (>99%). A few variable genomic regions exist, mainly at the ORF8 locus (coding for accessory proteins).

Zhou P, Yang XL, Wang XG, et al. **A pneumonia outbreak associated with a new coronavirus of probable bat origin.** Nature. 2020 Mar;579(7798):270-273. PubMed: <https://pubmed.gov/32015507>. Fulltext: <https://doi.org/10.1038/s41586-020-2012-7>

Full-length genome sequences from five patients at an early stage of the outbreak, showing 79.6% sequence identity to SARS-CoV and 96% to a bat coronavirus.

Genomic variation

MacLean O, Orton RJ, Singer JB, et al. **No evidence for distinct types in the evolution of SARS-CoV-2.** Virus Evolution. Full-text: <https://doi.org/10.1093/ve/veaa034>

Do not overinterpret genomic data! In this paper, authors discuss the difficulty in demonstrating the existence or nature of a functional effect of a viral mutation, and advise against overinterpretation.

Zhang X, Tan Y, Ling Y, et al. **Viral and host factors related to the clinical outcome of COVID-19.** Nature (2020). Full-text: <https://doi.org/10.1038/s41586-020-2355-0>

Viral variants do not affect outcome. This important study on 326 cases found at least two major lineages with differential exposure history during the early phase of the outbreak in Wuhan. Patients infected with these different clades did not exhibit significant differences in clinical features, mutation rates or transmissibility.

Origin and hosts

Andersen KG, Rambaut A, Lipkin WA, Holmes EC, Garry RF. **The proximal origin of SARS-CoV-2.** Nature Medicine. Published: 17 March 2020. Fulltext: <https://www.nature.com/articles/s41591-020-0820-9>

Review on notable genomic features of SARS-CoV-2, compared to alpha- and beta-coronaviruses. Insights on the origin, clearly showing that this virus is not a laboratory construct or a purposefully manipulated virus.

Cui J, Li F, Shi ZL. **Origin and evolution of pathogenic coronaviruses.** Nat Rev Microbiol. 2019 Mar;17(3):181-192. PubMed: <https://pubmed.gov/30531947>. Full-text: <https://doi.org/10.1038/s41579-018-0118-9>

SARS-CoV and MERS-CoV likely originated in bats, both jumping species to infect humans through different intermediate hosts.

Lam TT, Shum MH, Zhu HC, et al. **Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins.** Nature. 2020 Mar 26. PubMed: <https://pubmed.gov/32218527>. Fulltext: <https://doi.org/10.1038/s41586-020-2169-0>

Do Malayan pangolins act as intermediate hosts? Metagenomic sequencing identified pangolin-associated coronaviruses, including one with strong similarity to SARS-CoV-2 in the receptor-binding domain.

Xiao K, Zhai J, Feng Y, et al. **Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins.** Nature. 2020 May 7. PubMed: <https://pubmed.gov/32380510>. Full-text: <https://doi.org/10.1038/s41586-020-2313-x>

In a wildlife rescue center, authors found a coronavirus in 25 Malayan pangolins (some of them were very sick), showing 90-100% amino acid identity with SARS-CoV-2 in different genes. Comparative genomic analysis suggested that SARS-CoV-2 might have originated from the recombination of a Pangolin-CoV-like virus with a Bat-CoV-RaTG13-like virus. As the RBD of Pangolin-CoV is virtually identical to that of SARS-CoV-2, the virus in pangolins presents a potential future threat to public health. Pangolins and bats are both nocturnal animals, eat insects, and share overlapping ecological niches, which make pangolins the ideal intermediate host. *Stop the illegal pangolin trade!*

Zhang T, Wu Q, Zhang Z. **Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak.** Curr Biol. 2020 Mar 13. PubMed: <https://pubmed.gov/32197085>. Fulltext: <https://doi.org/10.1016/j.cub.2020.03.022>

This study suggests that pangolin species are a natural reservoir of SARS-CoV-2-like CoVs. Pangolin-CoV was 91.0% and 90.6% identical to SARS-CoV-2 and Bat-CoV RaTG13, respectively.

Zhou H, Chen X, Hu T, et al. **A Novel Bat Coronavirus Closely Related to SARS-CoV-2 Contains Natural Insertions at the S1/S2 Cleavage Site of the Spike Protein.** Curr Biol. 2020 May 11. PubMed: <https://pubmed.gov/32416074>. Full-text: <https://doi.org/10.1016/j.cub.2020.05.023>

A novel bat-derived coronavirus was identified from a metagenomics analysis of samples from 227 bats collected from Yunnan Province in 2019. Notably, RmYN02 shares 93.3% nucleotide identity with SARS-CoV-2 at the scale of the complete genome and 97.2% identity in the lab gene, in which it is the closest relative of SARS-CoV-2 reported to

date. However, RmYN02 showed low sequence identity (61.3%) in the receptor binding domain and might not bind to ACE2.

Stability and transmission of the virus

Chin AW, Chu JT, Perera MR, et al. **Stability of SARS-CoV-2 in different environmental conditions.** The Lancet Microbe 2020, April 02. Full-text: [https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247\(20\)30003-3/fulltext](https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247(20)30003-3/fulltext)

SARS-CoV-2 was highly stable at 4°C (almost no reduction on day 14) but sensitive to heat (70°C: inactivation 5 min, 56°: 30 min, 37°: 2 days). It also depends on the surface: No infectious virus could be recovered from print and tissue paper after 3 hours, from treated wood and cloth on day 2, from glass and banknotes on day 4, stainless steel and plastic on day 7. Strikingly, a detectable level of infectious virus (<0.1% of the original inoculum) was still present on the outer layer of a surgical mask on day 7.

Kim YI, Kim SG, Kim SM, et al. **Infection and Rapid Transmission of SARS-CoV-2 in Ferrets.** Cell Host Microbe. 2020 Apr 5. PubMed: <https://pubmed.gov/32259477>. Full-text: <https://doi.org/10.1016/j.chom.2020.03.023>.

Ferrets shed the virus in nasal washes, saliva, urine, and feces up to 8 days post-infection. They may represent an infection and transmission animal model of COVID-19 that may facilitate development of SARS-CoV-2 therapeutics and vaccines.

Leung NH, Chu Dk, Shiu EY. **Respiratory virus shedding in exhaled breath and efficacy of face masks.** Nature Med 2020, April 3. <https://doi.org/10.1038/s41591-020-0843-2>

This study from Hong Kong (performed 2013-16) quantified virus in respiratory droplets and aerosols in exhaled breath. In total, 111 participants (infected with seasonal coronavirus, influenza or rhinovirus) were randomized to wear or

not to wear a simple surgical face mask. Results suggested that masks could be used by ill people to reduce onward transmission. In respiratory droplets, seasonal coronavirus was detected in 3/10 (aerosols: 4/10) samples collected without face masks, but in 0/11 (0/11) from participants wearing face masks. Influenza viruses were detected in 6/23 (8/23) without masks, compared to 1/27 (aerosol 6/27!) with masks. For rhinovirus, there were no significant differences at all. Of note, authors also identified virus in some participants who did not cough at all during the 30 min exhaled breath collection, suggesting droplet and aerosol routes of transmission from individuals with no obvious signs or symptoms.

Shi J, Wen Z, Zhong G, et al. **Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2.** Science. 2020 Apr 8. PubMed: <https://pubmed.gov/32269068>. Full-text: <https://doi.org/10.1126/science.abb7015>

SARS-CoV-2 replicates poorly in dogs, pigs, chickens, and ducks. However, ferrets and cats are permissive to infection and cats were susceptible to airborne infection. But cat owners can relax. Experiments were done in a small number of cats exposed to high doses of the virus, probably more than found in real-life. It also remains unclear if cats secrete enough coronavirus to pass it on to humans.

van Doremalen N, Bushmaker T, Morris DH, et al. **Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1.** N Engl J Med. 2020 Mar 17. PubMed: <https://pubmed.gov/32182409>. Fulltext: <https://doi.org/10.1056/NEJMc2004973>

Stability of SARS-CoV-2 was similar to that of SARS-CoV-1, indicating that differences in the epidemics probably arise from other factors and that aerosol and fomite transmission of SARS-CoV-2 is plausible. The virus can remain viable and infectious in aerosols for hours and on surfaces up to days (depending on the inoculum shed).

Cell tropism, ACE expression

Chu H, Chan JF, Yuen TT, et al. **Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study.** *Lancet Microbe* April 21, 2020. Full-text: [https://doi.org/10.1016/S2666-5247\(20\)30004-5](https://doi.org/10.1016/S2666-5247(20)30004-5)

An elegant study, explaining distinct clinical features of COVID-19 and SARS. Investigation of cell susceptibility, species tropism, replication kinetics, and virus-induced cell damage from both SARS-CoVs, using live infectious virus particles. SARS-CoV-2 replicated more efficiently in human pulmonary cells, indicating that SARS-CoV-2 has most likely adapted better to humans. SARS-CoV-2 replicated significantly less in intestinal cells (might explain lower diarrhea frequency compared to SARS) but better in neuronal cells, highlighting the potential for neurological manifestations.

Hou YJ, Okuda K, Edwards CE, et al. **SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract.** *Cell*, May 26, 2020. Full-text: <https://doi.org/10.1016/j.cell.2020.05.042>

This study quantitated differences in ACE2 receptor expression and SARS-CoV-2 infectivity in the nose (high) vs the peripheral lung (low). If the nasal cavity is the initial site mediating seeding of the lung via aspiration, these studies argue for the widespread use of masks to prevent aerosol, large droplet, and/or mechanical exposure to the nasal passages.

Hui KPY, Cheung MC, Perera RAPM, et al. **Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures.** *Lancet Respir Med*. 2020 May 7. PubMed: <https://pubmed.gov/32386571>. Full-text: [https://doi.org/10.1016/S2213-2600\(20\)30193-4](https://doi.org/10.1016/S2213-2600(20)30193-4)

More insights into the transmissibility and pathogenesis. Using *ex vivo* cultures, the authors evaluated tissue and cel-

lular tropism of SARS-CoV-2 in human respiratory tract and conjunctiva in comparison with other coronaviruses. In the bronchus and in the conjunctiva, SARS-CoV-2 replication competence was higher than SARS-CoV. In the lung, it was similar to SARS-CoV but lower than MERS-CoV.

Shang J, Ye G, Shi K. **Structural basis of receptor recognition by SARS-CoV-2.** Nature 2020, March 30. Full-text: <https://doi.org/10.1038/s41586-020-2179-y>.

How well does SARS-CoV-2 recognize hACE2? Better than other coronaviruses. Compared to SARS-CoV and RaTG13 (isolated from bats), ACE2-binding affinity is higher. Functionally important epitopes in SARS-CoV-2 RBM are described that can potentially be targeted by neutralizing antibody drugs.

Sungnak W, Huang N, Bécavin C, et al. **SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes.** Nature Medicine, Published: 23 April 2020. Full-text: <https://www.nature.com/articles/s41591-020-0868-6>

Another elegant paper, confirming the expression of ACE2 in multiple tissues shown in previous studies, with added information on tissues not previously investigated, including nasal epithelium and cornea and its co-expression with TMPRSS2. Potential tropism was analyzed by surveying expression of viral entry-associated genes in single-cell RNA-sequencing data from multiple tissues from healthy human donors. These transcripts were found in specific respiratory, corneal and intestinal epithelial cells, potentially explaining the high efficiency of SARS-CoV-2 transmission.

Wang X, Xu W, Hu G, et al. **SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion.** Cell Mol Immunol. 2020 Apr 7. PubMed: <https://pubmed.gov/32265513>. Full-text: <https://doi.org/10.1038/s41423-020-0424-9>

It remains unclear whether SARS-CoV-2 can also infect T cells, resulting in lymphocytopenia. Using a model with pseudoviruses, authors showed that SARS-CoV-2 infects (but does not replicate in) T cells through S protein-mediated membrane fusion. T-cell lines were significantly more sensitive to SARS-CoV-2 infection when compared with SARS-CoV. Of note, a very low expression level of hACE2 was found, indicating that a novel receptor might mediate SARS-CoV-2 entry into T cells.

Spike protein

Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. **The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade.** Antiviral Res. 2020 Apr;176:104742. PubMed: <https://pubmed.gov/32057769>. Fulltext: <https://doi.org/10.1016/j.antiviral.2020.104742>

Identification of a peculiar furin-like cleavage site in the Spike protein of SARS-CoV-2, lacking in other SARS-like CoVs. Potential implication for the development of antivirals.

Watanabe Y, Allen JD, Wrapp D, McLellan JS, Crispin M. **Site-specific glycan analysis of the SARS-CoV-2 spike.** Science. 2020 May 4. PubMed: <https://pubmed.gov/32366695>. Full-text: <https://doi.org/10.1126/science.abb9983>

The surface of the envelope spike is dominated by host-derived glycans. These glycans facilitate immune evasion by shielding specific epitopes from antibody neutralization. SARS-CoV-2 S gene encodes 22 N-linked glycan sequons per protomer. Using a site-specific mass spectrometric approach, the authors reveal these glycan structures on a recombinant SARS-CoV-2 S immunogen.

Binding to ACE

Lan J, Ge J, Yu J, et al. **Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor**. Nature. Published: 30 March 2020. Full-text: <https://www.nature.com/articles/s41586-020-2180-5>

To elucidate the SARS-CoV-2 RBD and ACE2 interaction at a higher resolution/atomic level, authors used X-ray crystallography. Binding mode was very similar to SARS-CoV, arguing for a convergent evolution of both viruses. The epitopes of two SARS-CoV antibodies targeting the RBD were also analysed with the SARS-CoV-2 RBD, providing insights into the future identification of cross-reactive antibodies.

Wang Q, Zhang Y, Wu L, et al. **Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2**. Cell. 2020 Apr 7. PubMed: <https://pubmed.gov/32275855>. Full-text: <https://doi.org/10.1016/j.cell.2020.03.045>

Atomic details of the crystal structure of the C-terminal domain of SARS-CoV-2 spike protein in complex with human ACE2 are presented. The hACE2 binding mode of SARS-CoV-2 seems to be similar to SARS-CoV, but some key residue substitutions slightly strengthen the interaction and lead to higher affinity for receptor binding. Antibody experiments indicated notable differences in antigenicity between SARS-CoV and SARS-CoV-2

Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. **Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2**. Science. 2020 Mar 27;367(6485):1444-1448. PubMed: <https://pubmed.gov/32132184>. Full-text: <https://doi.org/10.1126/science.abb2762>

Using cryo-electron microscopy, this paper shows how SARS-CoV-2 binds to human cells. The first step in viral entry is the binding of the viral trimeric spike protein to the human receptor angiotensin-converting enzyme 2 (ACE2). The authors present the structure of human ACE2 in com-

plex with a membrane protein that it chaperones, B0AT1. The structures provide a basis for the development of therapeutics targeting this crucial interaction.

Cell entry

Hoffmann M, Kleine-Weber H, Schroeder S, et al. **SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.** Cell. 2020 Mar 4. PubMed: <https://pubmed.gov/32142651>. Fulltext: <https://doi.org/10.1016/j.cell.2020.02.052>

This work shows how viral entry happens. SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming. In addition, sera from convalescent SARS patients cross-neutralized SARS-2-S-driven entry.

Letko M, Marzi A, Munster V. **Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses.** Nat Microbiol. 2020 Apr;5(4):562-569. PubMed: <https://pubmed.gov/32094589>. Full-text: <https://doi.org/10.1038/s41564-020-0688-y>

Important work on viral entry, using a rapid and cost-effective platform which allows to functionally test large groups of viruses for zoonotic potential. Host protease processing during viral entry is a significant barrier for several lineage B viruses. However, bypassing this barrier allows several coronaviruses to enter human cells through an unknown receptor.

Ou X, Liu Y, Lei X, et al. **Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV.** Nat Commun. 2020 Mar 27;11(1):1620. PubMed: <https://pubmed.gov/32221306>. Fulltext: <https://doi.org/10.1038/s41467-020-15562-9>

More on viral entry and on (the limited) cross-neutralization between SARS-CoV and SARS-CoV-2.

Yuan M, Wu NC, Zhu X, et al. **A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV.** Science. 2020 Apr 3. PubMed: <https://pubmed.gov/32245784>. Full-text: <https://doi.org/10.1126/science.abb7269>

Insights into antibody recognition and how SARS-CoV-2 can be targeted by the humoral response, revealing a conserved epitope shared between SARS-CoV and SARS-CoV-2. This epitope could be used for vaccines and the development of cross-protective antibodies.

Zhang L, Lin D, Sun X, et al. **Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved alpha-ketoamide inhibitors.** Science. 2020 Mar 20. PubMed: <https://pubmed.gov/32198291>. Fulltext: <https://doi.org/10.1126/science.abb3405>

Description of the X-ray structures of the main protease (Mpro, 3CLpro) of SARS-CoV-2 which is essential for processing the polyproteins that are translated from the viral RNA. A complex of Mpro and an optimized protease α -ketoamide inhibitor is also described.

RNA-dependent RNA polymerase (RdRp)

Gao Y, Yan L, Huang Y, et al. **Structure of the RNA-dependent RNA polymerase from COVID-19 virus.** Science. 15 May 2020: Vol. 368, Issue 6492, pp. 779-782. Full-text: <https://doi.org/10.1126/science.abb7498>

Using cryogenic electron microscopy, the authors describe the structure of the RNA-dependent RNA polymerase, another central enzyme of the viral replication machinery. It is also shown how remdesivir and sofosbuvir bind to this polymerase. The authors determined a 2.9-angstrom-resolution structure of the RNA-dependent RNA polymerase (also known as nsp12), which catalyzes the synthesis of viral RNA, in complex with two cofactors, nsp7 and nsp8.

Hillen HS, Kokic G, Farnung L et al. **Structure of replicating SARS-CoV-2 polymerase.** Nature 2020. Full-text: <https://doi.org/10.1038/s41586-020-2368-8>
The cryo-electron microscopic structure of the SARS-CoV-2 RdRp in active form, mimicking the replicating enzyme. Long helical extensions in nsp8 protrude along the exiting RNA, forming positively charged 'sliding poles'. These sliding poles can account for the known processivity of the RdRp that is required for replicating the long coronavirus genome. A nice video provides an animation of the replication machine.

Animals and animal models

Bao L, Deng W, Huang B, et al. **The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice.** Nature. 2020 May 7. PubMed: <https://pubmed.gov/32380511>. Full-text: <https://doi.org/10.1038/s41586-020-2312-y>

In transgenic mice bearing human ACE2 and infected with SARS-CoV-2, the pathogenicity of the virus was demonstrated. This mouse model will be valuable for evaluating antiviral therapeutics and vaccines as well as understanding the pathogenesis of COVID-19.

Chan JF, Zhang AJ, Yuan S, et al. **Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility.** Clin Infect Dis. 2020 Mar 26. PubMed: <https://pubmed.gov/32215622>. Fulltext: <https://doi.org/10.1093/cid/ciaa325>

A readily available hamster model as an important tool for studying transmission, pathogenesis, treatment, and vaccination against SARS-CoV-2.

Chandrashekar A, Liu J, Martinot AJ, et al. **SARS-CoV-2 infection protects against rechallenge in rhesus macaques.** Science. 2020 May. PubMed: <https://pubmed.gov/32434946>. Full-text: <https://doi.org/10.1126/science.abc4776>

No re-infection in macaques. Following initial viral clearance, 9 rhesus macaques were re-challenged on day 35 with

the same doses of virus that were utilized for the primary infection. Very limited viral RNA was observed in BAL on day 1 after re-challenge, with no viral RNA detected at subsequent timepoints. These data show that SARS-CoV-2 infection induced protective immunity against re-exposure in nonhuman primates.

Halfman PJ, Hatta M, Chiba S, et al. **Transmission of SARS-CoV-2 in Domestic Cats.** NEJM May 13, 2020. Full-text:

<https://www.nejm.org/doi/full/10.1056/NEJMc2013400>

Three domestic cats were inoculated with SARS-CoV-2. One day later, an uninfected cat was cohoused with each of the inoculated cats. All six cats became infected and developed antibody titers but none showed any symptoms. Cats may be a silent intermediate host.

Rockx B, Kuiken T, Herfst S, et al. **Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model.** Science 17 Apr 2020. Full text:

<https://science.sciencemag.org/content/early/2020/04/16/science.abb7314>

Macaques may serve as a model to test therapeutic strategies. Virus was excreted from nose and throat in the absence of clinical signs, and was detected in type I and II pneumocytes *in foci* of diffuse alveolar damage and in ciliated epithelial cells of nasal, bronchial, and bronchiolar mucosae. In SARS-CoV infection, lung lesions were typically more severe, while they were milder in MERS-CoV infection, where virus was detected mainly in type II pneumocytes.

Munster VJ, Feldmann F, Williamson BN, et al. **Respiratory disease in rhesus macaques inoculated with SARS-CoV-2.** Nature 2020. Full-text:

<https://doi.org/10.1038/s41586-020-2324-7>

SARS-CoV-2 caused respiratory disease in 8 rhesus macaques, lasting 8-16 days. High viral loads were detected in swabs as well as in bronchoalveolar lavages. This “model”

recapitulates COVID-19, with regard to virus replication and shedding, the presence of pulmonary infiltrates, histological lesions and seroconversion.

Sia SF, Yan L, Chin AWH. et al. **Pathogenesis and transmission of SARS-CoV-2 in golden hamsters**. Nature 2020. Full-text: <https://doi.org/10.1038/s41586-020-2342-5>

In most cases, you don't need monkeys. Golden Syrian hamsters may also work. SARS-CoV-2 transmitted efficiently from inoculated hamsters to naïve hamsters by direct contact and via aerosols. Transmission via fomites in soiled cages was less efficient. Inoculated and naturally-infected hamsters showed apparent weight loss, and all animals recovered with the detection of neutralizing antibodies.

Sit TH, Brackman CJ, Ip SM et al. **Infection of dogs with SARS-CoV-2**. Nature 2020. Full-text: <https://www.nature.com/articles/s41586-020-2334-5>

Two out of fifteen dogs (one Pomeranian and one German Shepherd) from households with confirmed COVID-19 cases in Hong Kong were found to be infected. Both dogs remained asymptomatic but later developed antibody responses detected using plaque reduction neutralization assays. Genetic analysis suggested that the dogs caught the virus from their owners. It still remains unclear whether infected dogs can transmit the virus to other animals or back to humans.

Vaccine (see also Immunology)

Le TT, Andreadakis Z, Kumar A, et al. **The COVID-19 vaccine development landscape**. Nature reviews drug discovery. 09 April 2020. Full-text: <https://www.nature.com/articles/d41573-020-00073-5>.

Brief data-driven overview by seven experts. The conclusion is that efforts are unprecedented in terms of scale and speed and that there is an indication that vaccine could be available by early 2021. As of 8 April 2020, the global vaccine land-

scape includes 115 candidates, of which the 5 most advanced candidates have already moved into clinical development, including mRNA-1273 from Moderna, Ad5-nCoV from CanSino Biologics, INO-4800 from Inovio, LV-SMENP-DC and pathogen-specific aAPC from Shenzhen Geno-Immune Medical Institute. The race is on!

Callaway E. **The race for coronavirus vaccines: a graphical guide, Eight ways in which scientists hope to provide immunity to SARS-CoV-2.** Nature 2020, 28 April 2020. 580, 576-577. Full-text: <https://doi.org/10.1038/d41586-020-01221-y>

Fantastic graphic review on current vaccine development. Easy to understand, it explains different approaches such as virus, viral-vector, nucleic-acid and protein-based vaccines.

Zhu FC, Li YH, Guan XH. **Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial.** Lancet May 22, 2020. Full-text: [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(20\)31208-3/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31208-3/fulltext)

Open label Phase I trial of an Ad5 vectored COVID-19 vaccine, using the full-length spike glycoprotein. A total of 108 healthy adults aged between 18 and 60 years from Wuhan, China, were given three different doses. ELISA antibodies and neutralising antibodies increased significantly and peaked 28 days post-vaccination. Specific T cell response peaked at day 14 post-vaccination. Follow up is still short and authors are going to follow up the vaccine recipients for at least 6 months, so more data will be obtained. Of note, adverse events were relatively frequent, encompassing pain at injection sites (54%), fever (46%), fatigue (44%) and headache (39%). Phase II studies are underway.

Pathogenesis (see also Immunology)

Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. **Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19.** Cell May 15, 2020. Full-text: <https://doi.org/10.1016/j.cell.2020.04.026>

Incredible in-depth analysis of host response to SARS-CoV-2 and other human respiratory viruses in cell lines, primary cell cultures, ferrets, and COVID-19 patients. Data consistently revealed a unique and inappropriate inflammatory response to SARS-CoV-2 which is imbalanced with regard to controlling virus replication versus activation of the adaptive immune response. It is defined by low levels of type I and III interferons juxtaposed to elevated chemokines and high expression of IL-6. The authors propose that reduced innate antiviral defenses coupled with exuberant inflammatory cytokine production are the defining and driving features of COVID-19. Given this dynamic, treatments for COVID-19 have less to do with the IFN response and more to do with controlling inflammation.

Bordoni V, Sacchi A, Cimini E. **An inflammatory profile correlates with decreased frequency of cytotoxic cells in COVID-19.** Clinical Infectious Diseases 2020, May 15. Full-text: <https://doi.org/10.1093/cid/ciaa577>

The increase in inflammatory mediators is correlated with a reduction of innate and adaptive cytotoxic antiviral function. The authors found a lower perforin+ NK cell number in 7 intensive care unit (ICU) patients compared to 41 non-ICU patients, suggesting an impairment of the immune cytotoxic arm as a pathogenic mechanism.

Grifoni A, Weiskopf D, Ramirez SI, et al. **Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals.** Cell 2020. Full-text: <https://doi.org/10.1016/j.cell.2020.05.015>
Cellular response is a major knowledge gap. This important study identified circulating SARS-CoV-2-specific CD8 and CD4 T cells in 70-100% of 20 COVID-19 convalescent patients,

respectively. CD4 T cell responses to spike protein were robust and correlated with the magnitude of IgG titers. Of note, the authors detected SARS-CoV-2-reactive CD4 T cells in 40-60% of unexposed individuals, suggesting cross-reactive T cell recognition between circulating seasonal coronaviruses and SARS-CoV-2.

Li H, Liu L, Zhang D, et al. **SARS-CoV-2 and viral sepsis: observations and hypotheses.** Lancet. 2020 May 9;395(10235):1517-1520. PubMed: <https://pubmed.gov/32311318>. Full-text: [https://doi.org/10.1016/S0140-6736\(20\)30920-X](https://doi.org/10.1016/S0140-6736(20)30920-X)

Brief but nice review and several hypotheses about SARS-CoV-2 pathogenesis. What happens during the second week - when resident macrophages initiating lung inflammatory responses are unable to contain the virus after SARS-CoV-2 infection and when both innate and adaptive immune responses are inefficient to curb the viral replication so that the patient would recover quickly?

Shen B, Yi X, Sun Y, et al. **Proteomic and Metabolomic Characterization of COVID-19 Patient Sera.** Cell May 27, 2020. Full-text: <https://www.sciencedirect.com/science/article/pii/S0092867420306279>

Molecular insights into the pathogenesis of SARS-CoV-2 infection. The authors applied proteomic and metabolomic technologies to analyze the proteome and metabolome of sera from COVID-19 patients and several control groups. Pathway analyses and network enrichment analyses of the 93 differentially expressed proteins showed that 50 of these proteins belong to three major pathways, namely activation of the complement system, macrophage function and platelet degranulation. It was found that 80 significantly changed metabolites were also involved in the three biological processes revealed in the proteomic analysis.

Tay MZ, Poh CM, Rénia L et al. **The trinity of COVID-19: immunity, inflammation and intervention.** Nat Rev Immunol (2020). Full-text: <https://www.nature.com/articles/s41577-020-0311-8>

Brilliant overview of the pathophysiology of SARS-CoV-2 infection. How SARS-CoV-2 interacts with the immune system, how dysfunctional immune responses contribute to disease progression and how they could be treated.

Vabret N, Britton GJ, Gruber C, et al. **Immunology of COVID-19: current state of the science.** Immunity 2020, May 05. Full-text: [https://www.cell.com/immunity/fulltext/S1074-7613\(20\)30183-7](https://www.cell.com/immunity/fulltext/S1074-7613(20)30183-7)

Fantastic review on the current knowledge of innate and adaptive immune responses elicited by SARS-CoV-2 infection and the immunological pathways that likely contribute to disease severity and death.

Other key papers

Monto AS, DeJonge P, Callear AP, et al. **Coronavirus occurrence and transmission over 8 years in the HIVE cohort of households in Michigan.** J Infect Dis. 2020 Apr 4. PubMed: <https://pubmed.gov/32246136>. Full-text: <https://doi.org/10.1093/infdis/jiaa161>

It's not clear whether SARS-CoV-2 behaves like other human coronaviruses (hCoVs). A longitudinal surveillance cohort study of children and their households from Michigan found that hCoV infections were sharply seasonal, showing a peak for different hCoV types (229E, HKU1, NL63, OC43) in February. Over 8 years, almost no hCoV infections occurred after March.

Thao TTN, Labrousseau F, Ebert N, et al. **Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform.** Nature. 2020 May 4. PubMed: <https://pubmed.gov/32365353>. Full-text: <https://doi.org/10.1038/s41586-020-2294-9>

An important technical advance, enabling the rapid generation and functional characterization of evolving RNA virus variants. The authors show the functionality of a yeast-

based synthetic genomics platform to genetically reconstruct diverse RNA viruses (which are cumbersome to clone and manipulate due to size and instability). They were able to engineer and resurrect chemically-synthesized clones of SARS-CoV-2 only a week after receipt of the synthetic DNA fragments.

4. Immunology

Thomas Kamradt

Rapid progress is being made in deciphering the immune responses to SARS-CoV-2. Nevertheless, some of the important and most urgent questions remain unanswered including:

- Is someone who has recovered from COVID-19 protected from the disease?
- If yes, how long does the immune protection last?
- What are the correlates of protection?
- Why is the disease so much more severe in the elderly?
- How does the immune response against SARS-CoV-2 contribute to disease development? Are there pathogenic immune responses?
- Can we use immunological parameters to predict an individual patient's risk in developing severe disease?
- Can we develop a vaccine against SARS-CoV-2?

The current state of knowledge is beautifully summarised in a recent extensive review ([Vabret 2020](#)).

Protective antibodies

In the absence of robust experimental or clinical data on SARS-CoV-2-induced immune responses we can make some educated guesses based on prior experiences with endemic coronaviruses (e.g. 229E or OC43), the SARS-CoV and the MERS-CoV viruses. Experimental, serological and sero-epidemiological studies strongly suggest that coronaviruses, including SARS-CoV-2 induce neutralizing and protective antibodies. These studies also

seem to indicate that antibody-mediated protection is short-lived.

Cellular immune response

Less is known about cellular immune responses, i.e. T cell responses against coronaviruses. Experimental evidence from studies in mice suggests that T cells residing in the mucosa of the respiratory tract could be an important correlate of protection. However, although mice can be infected with coronaviruses including SARS-CoV, they do not develop the severe pulmonary symptoms that are characteristic of SARS and COVID-19. Therefore, these results have to be interpreted with caution. Human T cells from the respiratory mucosa of ill recovering humans would be necessary to clarify the issue but are difficult to come by.

These questions are not simply of an academic nature. Rational vaccine design is based on solid knowledge about protective immunity. As long as we do not know which protective immune response we need to induce by vaccination, vaccine development remains guesswork.

Important insights come from two recent studies on T cell responses against SARS-CoV-2 in healthy donors and COVID-19 patients. Alessandro Sette and coworkers examined T cell responses in COVID-19 patients and 11 healthy controls ([Grifoni 2020](#)). Using a pool of peptides from the SARS-CoV-2 S-, M-, N- and nsp proteins, they detected CD4+ and CD8+ T cell responses in 100% and 80% of the patients, respectively. Perhaps more surprisingly they also found reactive CD4+ and CD8+ T cells in 50% and 20% of unexposed healthy donors. In a similar study, Andreas Thiel and co-workers used large pools of overlapping peptides spanning the entire sequence of the SARS-CoV-2 S protein and detected S-reactive CD4+ T cells in 83% of the COVID-19 patients and 34% of the seronegative healthy donors ([Braun 2020](#)). The

high percentage of seronegative healthy donors who have SARS-CoV-2 reactive T cells is explained by previous exposure to other coronaviruses (“common cold” coronaviruses) which have proteins that are highly similar to those of SARS-CoV-2. Thiel and coworkers went on to show that T cells targeting the C-terminus of the S protein occur frequently in both patients and healthy donors, indicating cross-reactivity, whereas T cells targeting the N-terminus occur frequently in patients but not in healthy donors. The important question posed by these results is whether those frequently occurring cross-reactive T cells in healthy donors confer some degree of protection, are inconsequential or even potentially harmful if someone who possesses these cells becomes infected with SARS-CoV-2. Longitudinal studies will be needed to provide the answer and to determine how long after infection the SARS-CoV-2-specific T cells remain detectable in the blood of patients who have recovered from the disease.

The quest for a vaccine

The fundamentals:

- Recovery from infection often induces long-term and sometimes life-long immunity against the causative pathogen.
- Immunological memory protects against re-infection and is mediated by specific antibodies and T cells.
- Immunizations confer immunity without exposure to virulent pathogens. Immunization can be passive or active.
- In passive immunization protective antibodies are transferred from a donor to a recipient whereas active immunization induces a protective immune response in the recipient.

Passive immunization against SARS-CoV-2

Passive immunization against COVID-19 can be achieved with convalescent plasma, hyperimmune sera, or with neutralizing monoclonal antibodies.

Convalescent Plasma

Treatment of patients with convalescent plasma is based on the idea that someone who has recovered from an infection will have antibodies against the causative pathogen in their blood. Convalescent plasma is used for some infectious diseases including Argentinian hemorrhagic fever ([Casadevall 2004](#)). Prior experience shows antibody transfer is most effective when given prophylactically or early in the disease.

Convalescent plasma has been given to SARS patients. Regrettably, this was not done in the context of controlled clinical studies. A meta-analysis could therefore only conclude that the treatment was probably safe and perhaps helpful ([Mair-Jenkins 2015](#)). While drugs or vaccines against COVID-19 are still months or years away, convalescent plasma is available now.

To date, we do not know if all patients who have recovered from COVID-19 will harbor enough titers of neutralizing antibodies to confer protection upon transfer of plasma. Even the assays to determine the concentration of neutralizing antibodies are not standardized nor widely available.

Currently, convalescent plasma is given to COVID-19 patients (see Treatment chapter). Several randomized clinical studies are underway. The multicenter CONCOR-1 trial in Canada is due to start on April 27th with 1,200 participants planned and the CON-COVID trial in The Netherlands with a target number of more than 400 patients. These and similar studies will show if convalescent plasma is safe and effective.

Given the possibility of antibody-dependent disease enhancement (ADE), safety is an important consideration in these trials. One study on macaques found that passive transfer of anti-SARS-CoV-S immunoglobulin from immunized monkeys into naïve recipients resulted in acute lung injury after infection. The proposed mechanism was a diversion of macrophage activation from wound healing to pro-inflammatory (Liu 2019).

Enhanced lung-pathology upon antibody-transfer was also observed in a rabbit model of MERS (Houser 2017). Convalescent plasma has been given to MERS patients and one case-report raises the possibility of acute lung injury following convalescent plasma transfusion (Chun 2016).

Taken together, these data stress the necessity to administer convalescent plasma in controlled trials, which will determine safety and efficacy.

Pooled immunoglobulin preparations

Hyperimmune globulin preparations, e.g. cytomegalovirus immunoglobulin (CMVIG), pooled from many different donors, are currently the most frequently used form of passive antibody transfer. These preparations contain higher concentrations of pathogen-specific antibodies than convalescent plasma. However, they are more difficult to produce and there are currently no SARS-CoV-2 hyperimmune globulin preparations available.

Monoclonal antibodies

Neutralising monoclonal antibodies are a plausible therapeutic option against infectious diseases (Marston 2018). For example, a monoclonal antibody is licensed for prophylaxis against respiratory syncytial virus in at-risk infants. and mabs have been used to treat Ebola-patients (Marston 2018). Monoclonal antibodies against SARS-CoV have been tested in animal models and some were found to be effective. It is likely that mabs against SARS-

CoV-2 will soon be developed and tested. As explained above (see section on antibody dependent disease enhancement) the possibility of antibody dependent disease enhancement needs to be ruled out before such mabs can be applied in humans.

Active immunization against SARS-CoV-2

At the time of this writing, there are more than 100 COVID-19 vaccine candidates in different stages of preclinical development. Five candidate vaccines are in phase I clinical trials ([Thanh Le 2020](#)).

The speed of vaccine development is breathtaking. On 11 January, 2020 Chinese researchers published the sequence of the SARS-CoV-2 genome on the internet. Approximately 2 months later, on 16 March, an mRNA-based vaccine entered a phase I clinical trial. This was possible, thanks to knowledge gained in efforts to develop vaccines against SARS and MERS and the availability of innovative technologies.

Earlier work had identified the S protein of SARS-CoV and MERS-CoV as a suitable vaccine target. The S protein binds to its cellular receptor, ACE2, to infect human cells. A high degree of homology between the S proteins of the three viruses was quickly established after the discovery of SARS-CoV-2 and the interaction of SARS-CoV-2 S protein with ACE2 was confirmed. Thus, a vaccine target was identified in record time.

New technologies helped the rapid development of an mRNA-based vaccine. The principle was first used in 2013. The Chinese CDC had discovered H7N9, a novel avian influenza virus strain, and immediately published the sequence of the relevant antigens online. Synthetic biology approaches enabled the generation of a vaccine candidate within 8 days and that vaccine was shown to induce antibodies in mice ([Hekele 2013](#)).

Why, then, do we still wait for an effective and safe vaccine against SARS-CoV-2? There are still some obstacles to overcome.

Different strategies to develop a vaccine against SARS-CoV-2

Many fundamentally different strategies are currently used to develop a vaccine against COVID-19 ([Amanat 2020](#)).

The most traditional way to produce vaccines is the use of **whole viruses**, which are either *attenuated* or *inactivated*. Currently licensed examples include the vaccines against measles and yellow fever (attenuated virus) and influenza and polio (inactivated viruses). Efforts are ongoing to develop attenuated or inactivated SARS-CoV-2 as a vaccine.

Another approach is to use **recombinant viral proteins** as vaccine; licensed examples include the vaccines against hepatitis B and human papilloma virus. Efforts are ongoing to develop recombinant SARS-CoV-2 S protein as an immunogen.

A more recent approach is to use **recombinant viral vectors** in which a relevant antigen of the pathogenic virus is expressed. The only currently licensed example is the vaccine against Ebola, which is based on a modified vesicular stomatitis virus. An adenovirus-based recombinant vaccine against COVID-19 has entered a clinical phase I trial in March 2020.

DNA vaccines targeting the S protein are also in preclinical development. There are currently no licensed DNA vaccines, which might make the licensing process slower as compared with e.g. protein-based vaccines. A DNA vaccine against COVID-19 entered a clinical phase I trial in April 2020.

An **mRNA vaccine** targeting the S protein has been used in a clinical phase I trial that started on 16 March. There are currently no licensed mRNA vaccines, which might make the licensing process slower as compared with e.g. protein-based vaccines.

A vaccine based on genetically modified dendritic cells expressing a lentivirally encoded SARS-CoV-2 minigene and a study using genetically modified artificial antigen presenting cells entered a clinical phase I trial in March. There are currently no licensed vaccines based on **genetically modified antigen-presenting cells**, which might again make the licensing process slower as compared with e.g. protein-based vaccines.

While it is much too early to make any predictions on the safety, immunogenicity and efficacy of the many vaccines currently under development, it is useful to see what can be learned from prior attempts to develop vaccines against coronaviruses.

Vaccines against coronaviruses can induce pathological immune responses.

Rarely, vaccines can enhance disease rather than protect from disease ([Openshaw 2001](#)). Vaccines are administered to healthy people. SARS-CoV-2 causes a mild, if not clinically inapparent disease in at least 80% of those who are infected. Therefore, safety considerations are of utmost importance. Unfortunately, there is some data hinting at the possibility that the development of a safe vaccine against COVID-19 might be unusually difficult.

Vaccine-induced immune response against FIPV is harmful in kittens

Feline infectious peritonitis (FIP) is a severe and often fatal disease in cats. It is caused by a coronavirus, FIPV. Different attempts at vaccine development have failed. In an early study kittens that were vaccinated with an avirulent FIPV strain were more susceptible to infection with virulent FIPV than the non-vaccinated controls ([Pedersen 1983](#)). More worryingly were the results of a later study in which cats were immunized with a recombinant vaccinia virus that expressed the FIPV S protein. Vac-

cination induced low titers of neutralizing antibodies. Upon FIPV-challenge the previously immunized animals were not protected but died earlier than the controls (Vennema 1990). It is thought that antibody-mediated infection of macrophages and the deposition of immune complexes cause the more severe disease in immunized animals (Perlman 2005, Weiss 1981).

Immunopathology seen in experimental vaccines against SARS

Immunopathological or disease-enhancing effects were reported by many different research groups using different technologies and different animal models in an effort to develop a vaccine against SARS.

Immunization with recombinant modified vaccinia virus Ankara (rMVA) expressing the SARS-CoV spike (S) protein causes severe hepatitis in ferrets.

Ferrets are susceptible to SARS-CoV infection. Weingartl and colleagues immunized ferrets with recombinant modified vaccinia virus Ankara (rMVA) expressing the SARS-CoV S protein (Weingartl 2004). Upon challenge with the virus, high titers of neutralizing antibodies were detectable more rapidly in the immunized animals than in the controls. However, the ferrets immunized with rMVA-S developed severe hepatitis which was not the case in the control animals (Weingartl 2004). Ferrets are also highly susceptible to SARS-CoV-2 infection (Kim 2020) and are thus suitable for the evaluation of the safety of future vaccine candidates.

Immunization of mice results in type 2 inflammatory responses in the lungs

A group from North Carolina, USA used inactivated virus with or without adjuvant to immunize mice against SARS-CoV (Bolles 2011). The vaccine protected young and to a lesser extent, older

animals from morbidity and mortality following high-dose viral challenge. However, challenge with a heterologous virus resulted in inflammatory infiltrates and pulmonary eosinophilia that were more severe in the vaccinated animals. Moreover, in old mice the vaccine did not confer protection but still resulted in inflammatory infiltrates in the lung. The occurrence of lung immunopathology with this vaccine was later confirmed and extended by another group (Tseng 2012). Eosinophilic lung infiltrates were also observed when a recombinant baculovirus expressed S protein or coronavirus-like particles (VLPs) expressing the SARS-CoV S protein were used to immunize mice (Lokugamage 2008, Tseng 2012). It is important to note that these were mainly histopathological findings and the vaccinated mice had reduced viral titers upon challenge. However, these histopathological findings are reminiscent of those that were associated with vaccine-induced pathology in children that had received a vaccine against respiratory syncytial virus (RSV) in the 1960s (Castilow 2007). Moreover, lung pathology and even pneumonia were reported when mice were immunized with recombinant vaccinia virus (VV) expressing SARS-CoV S and nucleocapsid (N) proteins (Yasui 2008). Lung pathology was also observed when Venezuelan equine encephalitis virus replicon particles (VRP) expressing the N protein were used to immunize mice (Deming 2006).

Unfortunately, if perhaps not surprisingly, similar findings were reported for MERS-CoV vaccine candidates. An inactivated MERS-CoV vaccine induced neutralizing antibodies in mice and also resulted in an enhanced type 2 pathology in the lung, i.e. eosinophilic infiltrates and increased concentrations of IL-5 and IL-13 (Agrawal 2016).

Some studies suggest that this type 2 pathology may be ameliorated or prevented by using toll-like receptor agonists (Iwata-Yoshikawa 2014) or delta inulin (Honda-Okubo 2015) as adju-

vants for inactivated whole virus or recombinant spike protein vaccine candidates.

Together, these findings cause concern. Careful histopathological evaluation of the lungs should be part of the pre-clinical development of COVID-19 vaccines.

Immunization of non-human primates results in severe acute lung injury

In a recent study Chinese macaques were vaccinated with a modified vaccinia Ankara (MVA) virus encoding full-length SARS-CoV S glycoprotein (ADS-MVA) and challenged with SARS-CoV 8 weeks later (Liu 2019). Vaccination induced high levels of antibodies and reduced virus loads. However, the vaccinated monkeys had diffuse alveolar damage (DAD) (Liu 2019). An earlier study had used inactivated SARS-CoV to vaccinate four macaques. Three monkeys were protected upon challenge whereas one macaque had lung-pathology consistent with antibody-dependent disease enhancement (ADE) (Wang 2016). These authors further suggested that ADE was mediated by antibodies against certain epitopes of SARS-CoV S but not others (Wang 2016).

Anti-S antibodies enhance infection of human immune cells

Antibodies against SARS-CoV spike protein can enhance virus entry into human cells by interaction with conformational epitopes in the ACE2-binding domain (Yang 2005). Anti-Spike immune serum was reported to promote the infection of human hematopoietic cell lines by SARS-CoV. Virus entry was not mediated via ACE2 but depended on Fcγ receptor II (Jaume 2011). While the *in vivo* relevance of these findings remains to be determined, they add to the list of concerns that need to be addressed in the development of safe and effective vaccines against COVID-19.

Outlook

Given the massive and diverse ongoing efforts to develop a vaccine against COVID-19, we can be optimistic that a safe and effective vaccine will be available in the not-too-distant future. The development of a vaccine against Ebola took five years and there is reason to believe that the COVID-19 vaccine(s) will be developed even faster than that. We need to keep in mind that vaccine discovery and early development only require 30% of all the work and time required to bring a vaccine to the end user.

One challenge for the developers of COVID-19 vaccine(s) is that the elderly are most susceptible to the infection and carry a particularly high risk for severe or lethal disease. Due to immunosenescence, the elderly are notoriously difficult to immunize, requiring higher doses or particular immunization schemes in order to generate a protective immune response. Studies in mice indicate that older animals are also more likely to develop immunopathology upon vaccination.

A lesson that should have been learned already following the SARS outbreak is that more enzootic viruses will jump from their animal reservoirs to humans. Given the fact that not too many different viruses can cause severe and potentially deadly respiratory infections we should not stop our efforts once a SARS-CoV-2 specific vaccine is available. Instead, efforts should be made to develop a vaccine platform that can quickly be adapted to newly emerging coronaviruses. We do not know the date of the next outbreak, but we can be sure that SARS-CoV-2 is not the last coronavirus humankind will confront.

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5. Prevention

A thorough discussion of SARS-CoV-2 prevention will be presented in the 5th edition of COVID Reference by [Stefano Lazzari](#). In the meantime, please find this outline with key topics and references. At present, based on the current understanding of SARS-CoV-2 transmission presented in Chapter 2, several prevention measures can be considered at the personal, institutional, community and societal levels:

1. Personal infection prevention measures
 - Hand hygiene (washing or disinfecting)
 - Practice good respiratory hygiene/cough etiquette.
 - Use of face masks
 - Physical and social distancing. Avoid crowded places
 - Speak quietly, don't shout (or sing)!
 - Strengthen overall personal health and immunity
 - Household hygiene
 - Self-quarantine at home
 - Physical distancing
 - Use chemoprophylaxis (not yet available)
2. Preventive measures at community and social levels
 - Mandatory face masks
 - Ban mass gatherings (sports events, discos, crowded bars, religious celebrations etc.)
 - Localized and nationwide lockdowns
 - Travel bans/border closures
 - Widespread testing, intensive contact tracing (with or without smartphone apps)
 - Quarantine and isolation of suspected or confirmed cases

- Environmental hygiene and disinfection
 - Vaccinate for seasonal influenza and for COVID-19 (not yet available)
3. Preventive measures at institutional level
- Hospitals
 - Nursing facilities
 - Long-term Care Institutions
 - Workplaces
 - Schools
 - Prisons
 - Homeless shelters

Prevention at the personal level

Good respiratory hygiene/cough etiquette.

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Hand Hygiene

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Face masks

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Physical/Social distancing and avoiding crowded conditions

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- Asadi S, Wexler AS, Cappa CD, Barreda S, Bouvier NM, Ristenpart WD. **Aerosol emission and superemission during human speech increase with voice loudness.** Sci Rep. 2019 Feb 20;9(1):2348. PubMed: <https://pubmed.gov/30787335>. Full-text: <https://doi.org/10.1038/s41598-019-38808-z>

Household hygiene

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Chemoprophylaxis

Post-exposure prophylaxis (PEP) with antiviral drugs after documented exposure can reduce the risk of infection. In the future, SARS-CoV-2-PEP could be used to reduce viral shedding in suspected cases and as a prophylactic treatment of contacts.

Prevention at the community/societal levels

Mandatory face masks

- **Recommendation Regarding the Use of Cloth Face Coverings, Especially in Areas of Significant Community-Based Transmission,** US CDC 2020
- **WHO Advice on the use of masks in the context of COVID-19,** Interim guidance, 5 June 2020
- **European Centre for Disease Prevention and Control. Using face masks in the community.** Stockholm: ECDC 2020

Ban on mass gatherings

- McCloskey B, Zumla A, Ippolito G, et al. **Mass gathering events and reducing further global spread of COVID-19: a political and public health dilemma.** Lancet. 2020 Apr 4;395(10230):1096-1099. PubMed: <https://pubmed.gov/32203693>. Full-text: [https://doi.org/10.1016/S0140-6736\(20\)30681-4](https://doi.org/10.1016/S0140-6736(20)30681-4)
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Travel bans/border closures

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Quarantine and isolation of suspected or confirmed cases

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Environmental hygiene and disinfection

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Vaccinate for seasonal influenza and for COVID-19 (not yet available)

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Prevention at the institutional level

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Nursing facilities

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Long-term Care Institutions

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Workplaces

- **Prevention and Mitigation of COVID-19 at Work ACTION CHECKLIST**, International Labor Organization 16 April 2020
- **Guidance on Preparing Workplaces for COVID-19**, US CDC and OSHA 3990-03 2020.

Schools

- UK Department of Education **Guidance Actions for schools during the coronavirus outbreak** Updated 3 June 2020
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Prisons

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Homeless shelters

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6. Diagnostic Tests and Procedures

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Diagnosis

Rapid identification and isolation of infected individuals is crucial. Diagnosis is made using clinical, laboratory and radiological features. As symptoms and radiological findings of COVID-19 are non-specific, SARS-CoV-2 infection has to be confirmed by nucleic acid-based polymerase chain reaction (PCR), amplifying a specific genetic sequence in the virus. Within a few days after the first cases were published, a validated diagnostic workflow for SARS-CoV-2 was presented ([Corman 2020](#)), demonstrating the enormous response capacity achieved through coordination of academic and public laboratories in national and European research networks.

There is an interim guidance for laboratory testing for coronavirus disease (COVID-19) suspected human cases, published by WHO on March 19, 2020 ([WHO 2020](#)). Several comprehensive up-to-date reviews of laboratory techniques in diagnosing SARS-CoV-2 have been published recently ([Chen 2020](#), [Loeffelholz 2020](#)).

In settings with limited resources, no testing capacity should be wasted. Importantly, patients should only be tested if a positive test results in imperative action. This is not the case in the following examples:

- Young people who had contact with an infected person a few days earlier, have mild or moderate symptoms and live alone. They do not need PCR testing, even if they get fever. They'll remain in at-home quarantine, on sick leave if necessary, until at least 14 days after the onset of symptoms. A test would

only be useful to clarify whether they can work in a hospital or other health care facilities after quarantine. Some authorities require at least one negative test (nasopharyngeal) before starting work again (in addition to at least 48 hours of being symptom-free).

- A couple returning from an epidemic hotspot and feel a slight scratch in their throats. As they should remain in quarantine anyway, again, no testing is needed.
- A family of four with typical COVID-19 symptoms. Testing only one (symptomatic) person is sufficient. If the test is positive, it is not necessary to test the other household contacts – as long as they stay at home.

These decisions are not easy to communicate, particularly to fearful and worried patients.

In other situations, however, a test must be immediately carried out and repeated if necessary, especially for medical professionals with symptoms, but also, for example, in nursing homes, in order to detect an outbreak as quickly as possible.

Even though there are constantly updated recommendations by authorities and institutions of the country's health system about who should be tested by whom and when: they are constantly changing and have to be constantly adapted to the local epidemiological situation. With decreasing infection rates and increasing test capacities, more patients will certainly be able to be tested in the future, and the indication for a test will be expanded.

Specimen collection

SARS-CoV-2 can be detected in different tissues and body fluids. In a study on 1,070 specimens collected from 205 patients with COVID-19, bronchoalveolar lavage fluid specimens showed the highest positive rates (14 of 15; 93%), followed by sputum (72 of

104; 72%), nasal swabs (5 of 8; 63%), fibrobronchoscopy brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; 32%), feces (44 of 153; 29%), and blood (3 of 307; 1%). None of the 72 urine specimens tested positive (Wang X 2020). The virus was also not found in the vaginal fluid of 10 women with COVID-19 (Saito 2020).

It was also not found in two early studies on sperm and breast milk (Song 2020, Scorzoloni 2020). However, in a recent case report, SARS-CoV-2 RNA was detected in breast milk samples from an infected mother on 4 consecutive days. Detection of viral RNA in milk coincided with mild COVID-19 symptoms and a SARS-CoV-2 positive diagnostic test of the newborn (Groß 2020). On rare occasions, however, the virus may be also detected in tears and conjunctival secretions (Xia 2020).

Besides nasopharyngeal swabs, samples can be taken from sputum (if producible), endotracheal aspirate, or bronchoalveolar lavage. It is likely that lower respiratory samples are more sensitive than nasopharyngeal swabs. Especially in seriously ill patients, there is often more virus in the lower than in the upper respiratory tract (Huang 2020). However, there is always a high risk of “aerosolization” and thus the risk that staff members become infected.

However, viral replication of SARS-CoV-2 is very high in upper respiratory tract tissues which is in contrast to SARS-CoV (Wolfel 2020). According to WHO, respiratory material for PCR should be collected from upper respiratory specimens (nasopharyngeal and oropharyngeal swab or wash) in ambulatory patients (WHO 2020). It is preferred to collect specimens from both nasopharyngeal and oropharyngeal swabs which can be combined in the same tube.

Nasopharyngeal swabs – practical issues

It is important to carry out the swab correctly. Both nasopharynx and oropharyngeal swabs have a number of error options that all can lead to false negative results. In addition, protective measures must be taken in order not to endanger the examiner. Every swab carries a high risk of infection! Respiratory protection, protective glasses, gowns and gloves are required. The correct putting on and taking off of the protective clothing should be practiced! Many mistakes occur even when a protective mask is removed. There is a very useful video on protection, preparation, equipment, handling, removing personal protective equipment, etc ([Marty 2020](#)).

For the smear, the patient should sit on a chair and put his head slightly back. The examiner should stand at a slightly offset position in order to avoid a possible cough drop. Tell the patient that it can be uncomfortable for a short time. Swabs should be used that are suitable for virus detection and have the most flexible plastic shaft possible. Wooden sticks can inactivate viruses and pose a high risk of injury. The swab should be held between thumb and forefinger, like a pencil, so the end should not touch anything. The posterior wall of the nasopharynx is often reached after 5-7 cm, indicated by a slight resistance. “Nose popples” is not enough! Touching the teeth and tongue should be avoided when taking a throat swab; the swab should be removed from the back wall, directly next to the uvula. Caution with the gag reflex! There is a wealth of practical videos on the internet for the correct execution of the swabs. After appropriate instruction, many patients can perform the swabs themselves.

We have established swabs for patients who are able to do this (most of them!) at home. A courier with the tubes is sent directly to the patient's home, and the courier places the tubes in front of the door. Direct contact between patient and courier should be avoided. The swab tubes should not be touched by the courier

(either put them directly in a bag or collect them with an inverted bag) and should be brought back directly (no mailing!). This requires prior, precise instruction, but is usually quite feasible.

The swabs can be stored dry or in a small amount of NaCl solution; if necessary, this should be clarified with the laboratory beforehand. Quick PCR examination is important, preferably on the same day if possible. Heat is not favorable. In a small study, samples were inactivated by incubation in a water bath at 56°C for 30 minutes. 7/15 samples with low viral values converted to false negative. Longer storage also led to false negative results (Pan 2020).

Lower respiratory specimens may include sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease. However, a high risk of aerosolization should be considered (adhere strictly to infection prevention and control procedures). Additional clinical specimens may be collected as COVID-19 virus has been detected in blood and stool (see below).

Gathering specimens from nasopharyngeal and throat swabs can cause discomfort for patients and put health-care workers at risk. In contrast to many respiratory viruses, SARS-CoV-2 is present in saliva and several studies have shown that posterior oropharyngeal (deep throat) saliva samples are feasible and more acceptable to patients and healthcare workers (To 2020, Yu 2020). Throat washing may be used for monitoring due to its non-invasiveness and reliability. Throat washing was harvested by asking patients to oscillate over the posterior pharyngeal wall with 20 ml sterile normal saline. After 5-10 seconds, they spit out the normal saline from their throat to a sterile container. In 24 paired throat washings and nasopharyngeal swabs specimens, the positive testing rate of throat washing was much higher than that of swabs (Guo WL 2020).

Fecal shedding

Although no cases of transmission via fecal-oral route have yet been reported, there is also increasing evidence that SARS-CoV-2 is actively replicating in the gastrointestinal tract. Several studies showed prolonged presence of SARS-CoV-2 viral RNA in fecal samples (Chen 2020, Wu 2020). Combining results of 26 studies, a rapid review revealed that 54% of those patients tested for fecal RNA were positive. Duration of fecal viral shedding ranged from 1 to 33 days after a negative nasopharyngeal swab (Gupta 2020).

These studies have raised concerns about whether patients with negative pharyngeal swabs are truly virus-free, or sampling of additional body sites is needed. However, the clinical relevance of these finding remains unclear and there is one study that did not detect infectious virus from stool samples, despite having high virus RNA concentrations (Wolfel 2020). Therefore, the presence of nucleic acid alone cannot be used to define viral shedding or infection potential (Atkinson 2020). For many viral diseases including SARS-CoV or MERS-CoV, it is well known that viral RNA can be detected long after the disappearance of infectious virus.

Blood

SARS-CoV-2 is rarely detected in blood (Wang W 2020, Wolfel 2020). What about transmission risk associated with transfusions? In a screening study of 7,425 blood donations in Wuhan, plasma samples were found positive for viral RNA from 2 asymptomatic donors (Chang 2020).

Another study from Korea found seven asymptomatic blood donors who were later identified as COVID-19 confirmed cases. None of 9 recipients of platelets or red blood cell transfusions tested positive for SARS-CoV-2 RNA. Transfusion transmission of SARS-CoV-2 was considered to be unlikely (Kwon 2020). As with

feces, it remains unclear whether detectable RNA in the blood signifies infectivity.

PCR

Several different qPCR-based detection kits are available as labs worldwide have customized their PCR tests for SARS-CoV-2, using different primers targeting different sections of the virus's genetic sequence. A review of different assays and diagnostic devices was recently published (Loeffelholz 2020). A protocol for real-time (RT)-PCR assays for the detection of SARS-CoV-2 for two RdRp targets (IP2 and IP4) is described at https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2

Novel real-time RT-PCR assays targeting the RNA-dependent RNA polymerase (RdRp)/helicase, spike and nucleocapsid genes of SARS-CoV-2 may help to improve the laboratory diagnosis of COVID-19. Compared to the reported RdRp-P2 assay which is used in most European laboratories, these assays do not cross-react with SARS-CoV in cell culture and may be more sensitive and specific (Chan JF 2020).

If not, the limits of detection of six commercial kits differ substantially (up to 16-fold difference), with the poorest limits likely leading to false-negative results when RT-PCR were used to detect SARS-CoV-2 infection (Wang X 2020). According to the authors, manufacturers should analyze the existing problems according to the clinical application and further improve their products.

Qualitative PCR

A qualitative PCR ("positive or negative") is usually sufficient in routine diagnostics. Quantification of viral RNA is currently (still) only of academic interest.

False positive results are rare. However, they do occur. Though the analytical specificity of these tests is usually 100%, the clinical specificity is less, due to contamination (a significant problem for NAT procedures) and/or human error in the handling of samples or data (very hard to eliminate entirely). As seen with serology (see below), these false positive results will have substantial-to-large effects when prevalence is low (Andrew Cohen, personal communication).

Another problem of any qualitative PCR is false negative results which have many causes. Incorrect smears are particularly common, but laboratory errors also occur. In a review of 7 studies with a total of 1,330 respiratory samples the authors estimated the false-negative rate of RT-PCR by day since infection. Over the 4 days before symptom onset, the rate decreased from 100% to 67%. On the day of symptom onset (day 5), the rate was 38%, decreased to 20% (day 8) and then began to increase again, from 21% (day 9) to 66% (day 21). If clinical suspicion is high, infection should not be ruled out on the basis of RT-PCR alone. The false-negative rate is lowest 3 days after onset of symptoms, or approximately 8 days after exposure (Kucirka 2020). Figure 1 illustrates PCR and antibody detection during SARS

Several studies have shown that asymptomatic patients also have positive PCR results and can transmit the virus (Bai 2020, Cereda 2020, Rothe 2020). Viral shedding may begin 2 to 3 days before the appearance of the first symptoms. Analyzing a total of 414 throat swabs in 94 patients, the highest viral load in throat swabs was found at the time of symptom onset. Infectiousness started from 2.3 days (95% CI, 0.8–3.0 days) before symptom onset and peaked at 0.7 days before symptom onset (He 2020). Infectiousness was estimated to decline quickly within 7 days.

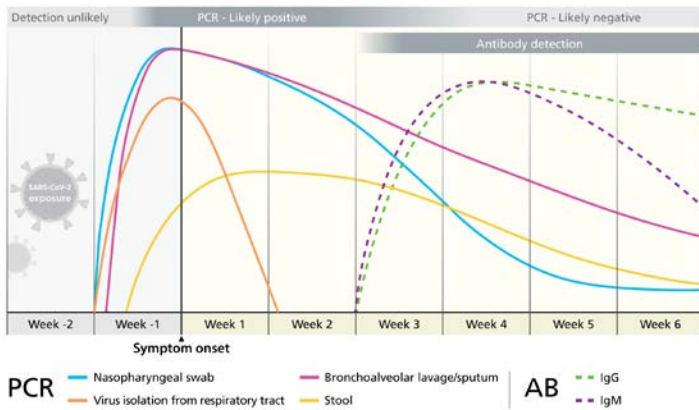


Figure 1. Timeline of diagnostic markers for detection of SARS-CoV-2. AB = Antibody.

In a cohort of 113 symptomatic patients, the median duration of detection of SARS-CoV-2 RNA was 17 days (interquartiles 13-22 days), measured from the onset of the disease. In some patients, the PCR was positive even longer: male gender and a severe course (invasive mechanical ventilation) were independent risk factors for prolonged shedding (Xu K 2020).

Recent reports from patients have repeatedly gained much media attraction, showing positive results after repeated negative PCR and clinical recovery (Lan 2020, Xiao AT 2020, Yuan 2020). These studies raise the question of re-activation or re-infection of COVID-19 (see below, clinical chapter). Currently, the results are much more likely due to methodological problems (Li 2020). At low virus levels, especially during the last days of an infection, the viral load can fluctuate and sometimes be detectable, sometimes not (Wölfel 2020). Reactivation, and also a rapid reinfection would be very unusual for coronaviruses.

Quantification of viral load

Several studies have evaluated the SARS-CoV-2 viral load in different specimens. In a small prospective study, the viral load in nasal and throat swabs obtained from 17 symptomatic patients was analyzed in relation to day-of-onset of any symptoms (Zou 2020). Of note, the viral load detected in the asymptomatic patients was similar to that in the symptomatic patients, which suggests the transmission potential of asymptomatic or minimally symptomatic patients.

In another study on 82 infected individuals, the viral loads in throat swab and sputum samples peaked at around 5–6 days after symptom onset, ranging from around 79,900 copies/ml in the throat to 752,000 copies per mL in sputum (Pan 2020). In a study on oropharyngeal saliva samples, unlike SARS, patients with COVID-19 had the highest viral load near presentation, which could account for the fast-spreading nature of this epidemic (To 2020). The median viral load in posterior oropharyngeal saliva or other respiratory specimens at presentation was 5.2 log₁₀ copies per mL (IQR 4.1–7.0) in this study. In a total of 323 samples from 76 patients, the average viral load in sputum (17,429 copies/test) was significantly higher than in throat swabs (2,552 copies) and nasal swabs (651 copies). Viral load was higher in the early and progressive stages than in the recovery stage (Yu 2020). According to a recently published study, viral shedding may already begin 2–3 days before the appearance of the first symptoms and the infectiousness profile may more closely resemble that of influenza than of SARS (He 2020).

Higher viral loads might be associated with severe clinical outcomes. In a study evaluating serial samples from 21 mild and 10 severe cases (Liu 2020), mild cases were found to have an early viral clearance, with 90% of these patients repeatedly testing negative on RT-PCR by day 10 post-onset. By contrast, all severe cases still tested positive at or beyond day 10 post-onset. Howev-

er, large and prospective trials are needed to evaluate the role of SARS-CoV-2 viral load as a marker for assessing disease severity and prognosis.

Should we measure the viral load? Probably yes. It may be helpful in clinical practice. A positive RT-qPCR result may not necessarily mean the person is still infectious or that they still have any meaningful disease. The RNA could be from non-viable virus and/or the amount of live virus may be too low for transmission. RT-qPCR provides quantification by first reverse transcribing RNA into DNA, and then performing qPCR where a fluorescence signal increases proportionally to the amount of amplified nucleic acid. The test is positive if the fluorescence reaches a specified threshold within a certain number of PCR cycles (Ct value, inversely related to the viral load). Many qPCR assays use a Ct cut-off of 40, allowing detection of very few starting RNA molecules. Some experts ([Tom 2020](#)) suggest using this Ct value or to calculate viral load which can help refine decision-making (shorter isolation etc). Unfortunately, there is still wide heterogeneity and inconsistency of the standard curves calculated from studies that provided Ct values from serial dilution samples and the estimated viral loads. According to other experts, precautions are needed when interpreting the Ct values of SARS-CoV-2 RT-PCR results shown in COVID-19 publications to avoid misunderstanding of viral load kinetics for comparison across different studies ([Han 2020](#)).

Test systems other than PCR

Point-of-care tests

Point-of-care tests are easy-to-use devices to facilitate testing outside of laboratory settings ([Joung 2020](#)). They are eagerly awaited. On May 6, the FDA granted an emergency use authorization for a clustered regularly interspaced short palindromic repeats (CRISPR)-based SARS-CoV-2 fluorescent assay marketed

by Sherlock Biosciences. This method gives results in an hour and has successfully diagnosed 12 positive and 5 negative COVID-19 patients, with at least 2 of 3 replicates scoring positive in infected persons. However, its use still remains limited to laboratories certified to perform high-complexity tests. On May 6, FDA has also authorized Quidel's Sofia 2 SARS Antigen Fluorescent Immunoassay. This test must be read on a dedicated analyzer and detects SARS-CoV-2 nucleocapsid protein from nasopharyngeal swabs in 15 min. According to the manufacturer, the assay demonstrated acceptable clinical sensitivity and detected 47/59 infections (80%). Unfortunately, no peer-reviewed papers have been published to date. Given the low sensitivity, these tests may mainly serve as an early tool to identify infectious individuals very rapidly, i.e. in the emergency unit. They will not work as a general diagnostic test.

Diagnosis in the setting of shortage of PCR test kits

There is no doubt that the overall goal must be to detect as many infections as possible. However, in many countries, a shortage of supply test kits does not meet the need of a growing infected population. Thus, pooled samples are often used to save material. Several samples are examined together. Only when such a pooled sample is positive, will the samples be examined individually.

Some studies have also investigated whether the diagnosis in high prevalence periods and countries cannot be made without PCR detection if necessary. A large retrospective case-control study from Singapore has evaluated predictors for SARS-CoV-2 infection, using exposure risk factors, demographic variables, clinical findings and clinical test results (Sun 2020). Even in the absence of exposure risk factors and/or radiologic evidence of pneumonia, clinical findings and tests can identify subjects at high risk of COVID-19. Low leukocytes, low lymphocytes, higher

body temperature, higher respiratory rate, gastrointestinal symptoms and decreased sputum production were strongly associated with a positive SARS-CoV-2 test. However, those preliminary prediction models are sensitive to the local epidemiological context and phase of the global outbreak. They make only sense during times of high incidence. In other words: if I see a patient during the peak of an epidemic, presenting with fever, cough, shortness of breath and lymphopenia, I can be almost sure that this patient suffers from COVID-19. During phases, when the incidence is lower, these models do not make sense. There is no doubt that the nucleic acid test serves as the gold standard method for confirmation of infection. Whenever PCR is available, PCR should be performed.

Serology (antibody testing)

Detection of past viral infections by looking for antibodies an infected person has produced will be among the most important goals in the fight against the COVID-19 pandemic. Antibody testing is multipurpose: these serological assays are of critical importance to determine seroprevalence, previous exposure and identify highly reactive human donors for the generation of convalescent serum as therapeutic. They will support contact tracing and screening of health care workers to identify those who are already immune. How many people really got infected, in how many did the virus escape the PCR diagnosis, and for what reasons, how many patients are asymptomatic, and what is the real mortality rate in a defined population? Only with comprehensive serology testing (and well-planned epidemiological studies) will we be able to answer these questions and reduce the ubiquitous undisclosed number in the current calculations. Several investigations are already underway in a wide variety of locations worldwide.

In recent weeks it has become clear that serology testing may also aid as a complementary diagnostic tool for COVID-19. The seroconversion of specific IgM and IgG antibodies were observed as early as the 4th day after symptom onset. Antibodies can be detected in the middle and later stages of the illness (Guo L 2020, Xiao DAT 2020). If a person with a highly suspicious COVID-19 remains negative by PCR testing and if symptoms are ongoing for at least several days, antibodies may be helpful and enhance diagnostic sensitivity.

However, antibody testing is not trivial. The molecular heterogeneity of SARS-CoV-2 subtypes, imperfect performance of available tests and cross-reactivity with seasonal CoVs have to be considered (reviews: Krammer 2020, Torres 2020).

Tests

Several groups are working towards producing these tests (Amanat 2020), some of them are already commercially available. A nice overview of the different platforms, including binding assays such as enzyme-linked immunosorbent assays (ELISAs), lateral flow assays, or Western blot-based assays is given by Krammer 2020. In addition, functional assays that test for virus neutralization, enzyme inhibition, or bactericidal assays can also inform on antibody-mediated immune responses. Many caveats and open questions with regard to antibody testing are also discussed.

Antibody testing usually focuses on antigens (proteins). In the case of SARS-CoV-2, different Enzyme-Linked Immunosorbent Assay (ELISA) kits based on recombinant nucleocapsid protein and spike protein are used (Loeffelholz 2020). The SARS-CoV-2 spike protein seems to be the best target. However, which part of the spike protein to use is less obvious and there is a lot hanging on the uniqueness of the spike protein. The more unique it is, the lower the odds of cross-reactivity with other coronaviruses—

false positives resulting from immunity to other coronaviruses. Cross reactivity to other coronaviruses can be challenging. So called confirmation tests (usually neutralization tests) can be used to reduce false positive testings.

Even with a very high specificity of 99% and above, especially in low-prevalence areas, the informative value is limited and a high rate of false positive tests can be assumed. An example: With a specificity of 99%, it is expected that one test out of 100 is positive. In a high prevalence setting, this is less relevant. However, if a person is tested in a low prevalence setting, the likelihood that a positive test is really positive (the positive predictive value, i.e. the number of really positive tests divided by the number of all positive tests) is low. In a population with a given prevalence of 1%, the predictive value would only be 50%! Current estimates from Iceland, a well-defined but unselected population, still have shown a relatively constant rate of around 0.8% in March 2020 ([Gudbjartsson 2020](#)). Even in apparently more severely affected countries, the infection rates are only slightly higher. If we assume an infection number of 183,000 (May 30) for Germany, a country with one of the world's largest number of infections, and assume that the number of undetected infections is about 5 times as high, then the prevalence in Germany is overall still around 1%. Almost every hundredth is infected, every second positive test would be false positive, even with a specificity of 99%. General antibody screening in the population will therefore produce a fairly high rate of false positive tests.

Average sensitivity and specificity of FDA-approved antibody tests is 84.9% and 98.6%, respectively. Given variable prevalence of COVID-19 (1%-15%) in different parts, statistically the positive predictive value will be as low as 30% to 50% in areas with low prevalence ([Mathur 2020](#)).

Indication in clinical practice

But, outside clinical studies: who should be tested now? Testing actually makes no sense for patients with a previous, proven COVID-19 disease. However, it can still be done if, for example, you want to validate a test. In addition to those involved in health care or working in other professions with a high risk of transmission, such testing can also be useful in order to identify possible contact persons retrospectively. However, we only measure antibodies when the testing result has consequences. Patients should be informed about the low positive predictive value, especially in those without any evidence of prior disease or exposition to COVID-19. In these patients, antibody testing is not recommended. Outside epidemiological hot spots, virtually everybody is still seronegative. If positive, the predictive value is too low.

Kinetics of antibodies

Serologic responses to coronaviruses are only transient. Antibodies to other human, seasonal coronaviruses may disappear even after a few months. Preliminary data suggest that the profile of antibodies to SARS-CoV-2 is similar to SARS-CoV (Xiao DAT 2020). For SARS-CoV, antibodies were not detected within the first 7 days of illness, but IgG titre increased dramatically on day 15, reaching a peak on day 60, and remained high until day 180 from when it declined gradually until day 720. IgM was detected on day 15 and rapidly reached a peak, then declined gradually until it was undetectable on day 180 (Mo 2006). As with other viruses, IgM antibodies occur somewhat earlier than IgG antibodies which are more specific. IgA antibodies are relatively sensitive but less specific (Okba 2020).

The first larger study on the host humoral response against SARS-CoV-2 has shown that these tests can aid to the diagnosis of COVID-19, including subclinical cases (Guo 2020). In this study,

IgA, IgM and IgG response using an ELISA-based assay on the recombinant viral nucleocapsid protein was analyzed in 208 plasma samples from 82 confirmed and 58 probable cases (Guo 2020). The median duration of IgM and IgA antibody detection were 5 days (IQR 3-6), while IgG was detected on day 14 (IQR 10-18) after symptom onset, with a positive rate of 85%, 93% and 78% respectively. The detection efficiency by IgM ELISA was higher than that of PCR after 5.5 days of onset of symptoms. In another study of 173 patients, the seroconversion rates (median time) for IgM and IgG were 83% (12 days) and 65% (14 days), respectively. A higher titer of antibodies was independently associated with severe diseases (Zhao 2020).

In some patients, IgG occurs even faster than IgM. In a study on seroconversion patterns of IgM and IgG antibodies, the seroconversion time of IgG antibody was earlier than IgM. IgG antibody reached the highest concentration on day 30, while IgM antibody peaked on day 18, but then began to decline (Qu J 2020). The largest study to date reported on acute antibody responses in 285 patients (mostly non-severe COVID-19). Within 19 days after symptom onset, 100% of patients tested positive for antiviral IgG. Seroconversion for IgG and IgM occurred simultaneously or sequentially. Both IgG and IgM titers plateaued within 6 days after seroconversion. The median day of seroconversion for both IgG and IgM was 13 days post-symptom onset. No association between plateau IgG levels and clinical characteristics was found (Long 2020).

Do all asymptomatic individuals develop antibodies? Probably not. Among five asymptomatic cases, only one generated SARS-CoV-2 specific antibody responses within the first 4 weeks (Yongchen 2020).

Taken together, antibody testing is not only an epidemiological tool. It also may help in the diagnosis. It will be seen in the coming months how the human antibody response to SARS-CoV-2

evolves over time and how this response and titres correlate with immunity. It is also conceivable that in some patients (e.g. those with immunodeficiency), the antibody response remains reduced.

Radiology

Chest computed tomography

Computed tomography (CT) can play a role in both diagnosing and assessment of disease extent and follow-up. Chest CT has a relatively high sensitivity for diagnosis of COVID-19 (Ai 2020, Fang 2020). However, around half of patients may have a normal CT during the first 1-2 days after symptom onset (Bernheim 2020). On the other hand, it became clear very early in the current pandemic that a considerable proportion of subclinical patients (scans done before symptom onset) may already have pathological CT findings (Chan 2020, Shi 2020). In some of these patients showing pathological CT findings evident for pneumonia PCR in nasopharyngeal swabs was still negative (Xu 2020). On the other hand, half of the patients who later develop CT morphologically visible pneumonia can still have a normal CT in the first 1-2 days after the symptoms appear (Bernheim 2020).

However, one should not overestimate the value of chest CT. The recommendation by some Chinese researchers to include CT as an integral part in the diagnosis of COVID-19 has led to harsh criticism, especially from experts in Western countries. The Chinese studies have been exposed to significant errors and shortcomings. In view of the high effort and also because of the risk of infection for the staff, many experts strictly reject the general CT screening in SARS-CoV-2 infected patients or in those with suspicion (Hope 2020, Raptis 2020). According to the recommendation of the British Radiology Society, which made attempts to incorporate CT into diagnostic algorithms for COVID-19 diagnos-

tics, the value of CT remains unclear – even if a PCR is negative or not available (Nair 2020, Rodrigues 2020). A chest CT should only be performed if complications or differential diagnoses are considered (Raptis 2020).

In blinded studies, radiologists from China and the United States have attempted to differentiate COVID-19 pneumonia from other viral pneumonia. The specificity was quite high, the sensitivity much lower (Bai 2020). A recent metaanalysis found a high sensitivity but low specificity (Kim 2020). The sensitivity of CT was affected by the distribution of disease severity, the proportion of patients with comorbidities, and the proportion of asymptomatic patients. In areas with low prevalence, chest CT had a low positive predictive value (1.5-30.7%).

If pathological, images usually show bilateral involvement, with multiple patchy or ground-glass opacities (GGO) with subpleural distribution in multiple bilateral lobes. Lesions may display significant overlap with those of SARS and MERS (Hosseiny 2020).

A systematic review of imaging findings in 919 patients found bilateral multilobar GGO with a peripheral or posterior distribution, mainly in the lower lobes and less frequently within the right middle lobe as the most common feature (Salehi 2020). In this review, atypical initial imaging presentation of consolidative opacities superimposed on GGO were found in a smaller number of cases, mainly in the elderly population. Septal thickening, bronchiectasis, pleural thickening, and subpleural involvement were less common, mainly in the later stages of the disease. Pleural effusion, pericardial effusion, lymphadenopathy, cavitation, CT halo sign, and pneumothorax were uncommon (Salehi 2020).

The evolution of the disease on CT is not well understood. However, with a longer time after the onset of symptoms, CT findings are more frequent, including consolidation, bilateral and peripheral disease, greater total lung involvement, linear

opacities, “crazy-paving” pattern and the “reverse halo” sign (Bernheim 2020). Some experts have proposed that imaging can be sorted into four different phases (Li M 2020). In the early phase, multiple small patchy shadows and interstitial changes emerge. In the progressive phase, the lesions increase and enlarge, developing into multiple GGOs as well as infiltrating consolidation in both lungs. In the severe phase, massive pulmonary consolidations and “white lungs” are seen, but pleural effusion is rare. In the dissipative phase, the GGOs and pulmonary consolidations were completely absorbed, and the lesions began to change into fibrosis.

In a longitudinal study analyzing 366 serial CT scans in 90 patients with COVID-19 pneumonia, the extent of lung abnormalities progressed rapidly and peaked during illness days 6-11 (Wang Y 2020). The predominant pattern of abnormalities after symptom onset in this study was ground-glass opacity (45-62%). As pneumonia progresses, areas of lesions enlarge and developed into diffuse consolidations in both lungs within a few days (Guan 2020).

Most patients discharged had residual disease on final CT scans (Wang Y 2020). Studies with longer follow-up are needed to evaluate long-term or permanent lung damage including fibrosis, as is seen with SARS and MERS infections. Pulmonary fibrosis is expected to be the main factor leading to pulmonary dysfunction and decline of quality of life in COVID-19 survivors after recovery. More research is needed into the correlation of CT findings with clinical severity and progression, the predictive value of baseline CT or temporal changes for disease outcome, and the sequelae of acute lung injury induced by COVID-19 (Lee 2020).

Of note, chest CT is not recommended in all COVID-19 patients, especially in those who are well enough to be sent home or those with only short symptomatic times (< 2 days). In case of COVID-19, a large number of patients with infection or suspected infec-

tion swarm into the hospital. Consequently, the examination workload of the radiology department increases sharply. Because the transmission route of SARS-CoV-2 is through respiratory droplets and close contact transmission, unnecessary CT scan should be avoided. An overview of the prevention and control of the COVID-19 epidemic in the radiology department is given by An et al.

Ultrasound, PET and other techniques

Some experts have postulated that lung ultrasound (LUS) may be helpful, since it can allow the concomitant execution of clinical examination and lung imaging at the bedside by the same doctor (Buonsenso 2020, Soldati 2020). Potential advantages of LUS include portability, bedside evaluation, safety and possibility of repeating the examination during follow-up. Experience especially from Italy with lung ultrasound as a bedside tool has improved evaluation of lung involvement, and may also reduce the use of chest x-rays and CT. A point scoring system is employed by region and ultrasound pattern (Vetrugno 2020). However, the diagnostic and prognostic role of LUS in COVID-19 is uncertain.

Whether there is any potential clinical utility of other imaging techniques such as 18F-FDG PET/CT imaging in the differential diagnosis of complex cases also remains unclear (Deng 2020, Qin 2020).

In patients with neurological symptoms, brain MRI is often performed. In 27 patients, the most common imaging finding was cortical signal abnormalities on FLAIR images (37%), accompanied by cortical diffusion restriction or leptomeningeal enhancement (Kandemirli 2020). However, the complex clinical course including comorbidities, long ICU stay with multidrug regimens, respiratory distress with hypoxia episodes can all act as confounding factors and a clear cause-effect relationship be-

tween COVID-19 infection and MRI findings will be hard to establish.

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7. Clinical Presentation

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After an average incubation time of around 5 days (range: 2-14 days), a typical COVID-19 infection begins with dry cough and low-grade fever (38.1–39°C or 100.5–102.1°F), often accompanied by diminishment of smell and taste. In most patients, COVID-19 remains mild or moderate and symptoms resolve within a week and patients typically recover at home. Around 10% of patients remain symptomatic through the second week. The longer the symptoms persist, the higher the risk of developing more severe COVID-19, requiring hospitalization, intensive care and invasive ventilation. The outcome of COVID-19 is often unpredictable, especially in older patients with comorbidities. The clinical picture ranges from completely asymptomatic to rapidly devastating courses.

In this chapter we discuss clinical presentation, including incubation period and asymptomatic patients, frequent and rare symptoms, laboratory findings and risk factors for severe disease. Radiological findings are described in the diagnostic chapter.

Incubation period

A pooled analysis of 181 confirmed COVID-19 cases with identifiable exposure and symptom onset windows estimated the median incubation period to be 5.1 days with a 95% CI of 4.5 to 5.8 days (Lauer 2020). The authors estimated that 97.5% of those who develop symptoms will do so within 11.5 days (8.2 to 15.6 days) of infection. Fewer than 2.5% of infected persons will show symptoms within 2.2 days, whereas symptom onset will occur

within 11.5 days in 97.5%. However, these estimates imply that, under conservative assumptions, 101 out of every 10,000 cases will develop symptoms after 14 days of active monitoring or quarantine. Another analysis of 158 confirmed cases outside Wuhan estimated a very similar median incubation period of 5.0 days (95 % CI, 4.4 to 5.6 days), with a range of 2 to 14 days (Linton 2020). In a detailed analysis of 36 cases linked to the first three clusters of circumscribed local transmission in Singapore, the median incubation period was 4 days with a range of 1-11 days (Pung 2020). Taken together, the incubation period of around 4-6 days is in line with that of other coronaviruses causing SARS or MERS (Virlogeux 2016).

Of note, the time from exposure to onset of infectiousness (latent period) may be shorter. There is little doubt that transmission of SARS-CoV-2 during the late incubation period is possible (Li 2020). In a longitudinal study, the viral load was high 2-3 days before the onset of symptoms, and the peak was even reached 0.7 days before the onset of symptoms. The authors of this *Nature Medicine* paper estimated that approximately 44% (95% CI 25-69%) of all secondary infections are caused by such presymptomatic patients (He 2020).

Asymptomatic cases

Understanding the frequency of asymptomatic patients and the temporal course of asymptomatic transmission will be very important for assessing disease dynamics. It is important to distinguish those patients who will remain asymptomatic during the whole time of infection and those in which infection is still too early to cause symptoms (presymptomatic).

While physicians need to be aware of asymptomatic cases, the true percentage is difficult to assess. The probably best data come from 3,600 people on board the cruise ship Diamond Princess (Mizumoto 2020) who became involuntary actors in a “well-

controlled experiment” where passengers and crew comprised an environmentally homogeneous cohort. Due to insufficient hygienic conditions, >700 people became infected while the ship was quarantined in the port of Yokohama, Japan. After systematic testing, 328 (51.7%) of the first 634 confirmed cases were found to be asymptomatic. Considering the varying of the incubation period between 5.5 and 9.5 days, the authors calculated the true asymptomatic proportion at 17.9% (Mizumoto 2020).

From a total of 565 Japanese citizens evacuated from Wuhan, the asymptomatic ratio was estimated to be 42% (Nishiura 2020). Of 279 close contacts to COVID-19 patients who became PCR positive, 63 (23%) remained asymptomatic throughout their infections. Of note, 29 patients had abnormal CT findings (Wang Y 2020). In a screening study conducted in Iceland, the number of patients testing positive for SARS-CoV-2 but without symptoms was 44%, although some of these may have been pre-symptomatic (Gudbjartsson 2020). In an observational cohort study of 199 infected patients in a residential treatment center in South Korea, the rate of asymptomatic patients was 26% (Noh 2020). The range of true asymptomatic patients in the studies published to date is still broad and may depend on the population which was analyzed.

Asymptomatic patients may transmit the virus (Bai 2020, Rothe 2020). In a study from Northern Italy viral loads in nasal swabs between asymptomatic and symptomatic subjects did not differ significantly, suggesting the same potential for transmitting the virus (Cereda 2020). Of 63 asymptomatic patients in Chongqing, 9 (14%) transmitted the virus to others (Wang Y 2020). In an outbreak in a long-term care facility, 13/23 residents who tested positive were asymptomatic or presymptomatic on the day of testing (Kimball 2020). In another skilled nursing facility, of 48 residents, 27 (56%) were asymptomatic at the time of testing positive. Of these, 24 subsequently developed symptoms, with medi-

an time to onset of 4 days (Arons 2020). There is some evidence that shedding of RNA and viral load is somewhat shorter in asymptomatic (not presymptomatic!) patients (Noh 2020, Yang 2020).

Taken together, these preliminary studies indicate that around 20-40% of all COVID-19 infected subjects may remain asymptomatic during their infection. But it may well be that we are still quite wrong. Only large-scale field studies on seroprevalence will be able to clarify the exact proportion.

Symptoms

A plethora of symptoms have been described in the past months, clearly indicating that COVID-19 is a complex disease, which in no way consists only of a respiratory infection. Many symptoms are unspecific so that the differential diagnosis encompasses a wide range of infections, respiratory and other diseases. However, different clusters can be distinguished in COVID-19. The most common symptom cluster encompasses the respiratory system: cough, sputum, shortness of breath, and fever. Other clusters encompass musculoskeletal symptoms (myalgia, joint pain, headache, and fatigue), enteric symptoms (abdominal pain, vomiting, and diarrhoea); and less commonly, a mucocutaneous cluster.

Fever, cough, shortness of breath

Symptoms occur in the majority of cases (for asymptomatic patients, see below). In early studies from China (Guan 2020, Zhou 2020), fever was the most common symptom, with a median maximum of 38.3 C; only a few had a temperature of > 39 C. The absence of fever seems to be somewhat more frequent than in SARS or MERS; fever alone may therefore not be sufficient to detect cases in public surveillance. The second most common

symptom was cough, occurring in about two thirds of all patients. Among survivors of severe COVID-19 (Zhou 2020), median duration of fever was 12.0 days (8-13 days) and cough persisted for 19 days (IQR 12-23 days). In a meta-analysis of COVID-19 in papers published until February 23, fever (88.7%), cough (57.6%) and dyspnea (45.6%) were the most prevalent clinical manifestations (Rodriguez-Morales 2020). In another review, the corresponding percentages were 88.5%, 68.6% and 21.9%, respectively (Li 2020).

Fever and cough do not distinguish between mild and severe cases nor do they predict the course of COVID-19 (Richardson 2020, Petrilli 2020). In contrast, shortness of breath has been identified as a strong predictor of severe disease in larger studies. In a cohort of 1,590 patients, dyspnea was associated with an almost two-fold risk for critical disease (Liang 2020) and mortality (Chen 2020). Others found higher rates of shortness of breath, and temperature of > 39.0 in older patients compared with younger patients (Lian 2020). In the Wuhan study on patients with severe COVID-19, multivariate analysis revealed that a respiratory rate of > 24 breaths per minute at admission was higher in non-survivors (63% versus 16%).

Within the last weeks, huge cohort data from countries outside China have been published. However, almost all data applies to patients who were admitted to hospitals, indicating selection bias towards more severe and symptomatic patients.

- Among 20,133 patients in the UK who were admitted to 208 acute care hospitals in the UK between 6 February and 19 April 2020, the most common symptoms were cough (69%), fever (72%), and shortness of breath (71%), showing a high degree of overlap (Docherty 2020).
- Among 5,700 patients who were admitted to any of 12 acute care hospitals in New York between March 1, 2020, and April 4, 2020, only 30.7% had fever of > 38 C. A respiratory rate of $>$

24 breaths per minute at admission was found in 17.3% (Richardson 2020).

- Among the first 1,000 patients presenting at the NewYork-Presbyterian/Columbia University (Argenziano 2019), the most common presenting symptoms were cough (73%), fever (73%), and dyspnea (63%).

Musculoskeletal symptoms

The cluster of musculoskeletal symptoms encompasses myalgia, joint pain, headache, and fatigue. These are frequent symptoms, occurring each in 15-40% of patients (Argenziano 2019, Docherty 2020, Guan 2020). Although subjectively very disturbing and sometimes foremost in the perception of the patient, these symptoms tell us nothing about the severity of the clinical picture. However, they are frequently overlooked in clinical practice, and headache merits special attention.

According to a recent review (Bolay 2020), headache is observed in 11-34% of hospitalized COVID-19 patients, occurring in 6-10% as presenting symptom. Significant features are moderate-severe, bilateral headache with pulsating or pressing quality in the temporo-parietal, forehead or periorbital region. The most striking features are sudden to gradual onset and poor response to common analgesics. Possible pathophysiological mechanisms include activation of peripheral trigeminal nerve endings by the SARS-CoV-2 directly or through the vasculopathy and/or increased circulating pro-inflammatory cytokines and hypoxia.

Gastrointestinal symptoms

Cell experiments have shown that SARS-CoV and SARS-CoV-2 are able to infect enterocytes (Lamers 2020). Active replication has been shown in both bats and human intestinal organoids (Zhou 2020). Fecal calprotectin as a reliable fecal biomarker allowing

detection of intestinal inflammation in inflammatory bowel diseases and infectious colitis, was found in some patients, provides evidence that SARS-CoV-2 infection instigates an inflammatory response in the gut (Effenberger 2020). These findings explain why gastrointestinal symptoms are observed in a subset of patients and why viral RNA can be found in rectal swabs, even after nasopharyngeal testing has turned negative. In patients with diarrhea, stool viral RNA was detected at higher frequency (Cheung 2020).

In the early Chinese studies, however, gastrointestinal symptoms were rarely seen. In a meta-analysis of 60 early studies comprising 4,243 patients, the pooled prevalence of gastrointestinal symptoms was 18% (95% CI, 12%-25%); prevalence was lower in studies in China than other countries. As with otolaryngeal symptoms, it remains unclear whether this difference reflects geographic variation or differential reporting. Among the first 393 consecutive patients who were admitted to two hospitals in New York City, diarrhea (24%), and nausea and vomiting (19%) were relatively frequent (Goyal 2020). Among 18,605 patients admitted to UK Hospitals, 29% of all patients complained of enteric symptoms on admission, mostly in association with respiratory symptoms; however, 4% of all patients described enteric symptoms alone (Docherty 2020).

Otolaryngeal symptoms (including anosmia)

Although upper respiratory tract symptoms such as rhinorrhea, nasal congestion, sneezing and sore throat are relatively unusual, it has become clear within a few weeks that anosmia and hyposmia are important signs of the disease (Luers 2020). Interestingly, these otolaryngological symptoms appear to be much more common in Europe than in Asia. However, it is still unclear whether this is a real difference or whether these complaints in the initial phase in China were not recorded well enough. There

is now very good data from Europe: The largest study to date found that 1,754/2,013 patients (87%) reported loss of smell, whereas 1,136 (56%) reported taste dysfunction. Most patients had loss of smell after other general and otolaryngologic symptoms (Lechien 2020). Mean duration of olfactory dysfunction was 8.4 days. Females seem to be more affected than males. The prevalence of self-reported smell and taste dysfunction was higher than previously reported and may be characterized by different clinical forms. Anosmia may not be related to nasal obstruction or inflammation. Of note, only two thirds of patients reporting olfactory symptoms and who had objective olfactory testing had abnormal results.

“Flu plus ‘loss of smell’ means COVID-19”. Among 263 patients presenting in March (at a single center in San Diego) with flu-like symptoms, loss of smell was found in 68% of COVID-19 patients (n=59), compared to only 16% in negative patients (n=203). Smell and taste impairment were independently and strongly associated with positivity (anosmia: adjusted odds ratio 11, 95%CI: 5-24). Conversely, sore throat was independently associated with negativity (Yan 2020).

Among a total of 18,401 participants from the US and UK who reported potential symptoms on a smartphone app and had undergone a SARS-CoV-2 test, the proportion of participants who reported loss of smell and taste was higher in those with a positive test result (65 vs 22%). A combination of symptoms, including anosmia, fatigue, persistent cough and loss of appetite was appropriate to identify individuals with COVID-19 (Menni 2020).

Taken together, otolaryngeal symptoms do not indicate severity but are important indicators for SARS-CoV-2 infection.

Cardiovascular symptoms and issues

There is growing evidence of direct and indirect effects of SARS-CoV-2 on the heart, especially in patients with pre-existing heart

diseases (Bonow 2020). SARS-CoV-2 has the potential to infect cardiomyocytes, pericytes and fibroblasts via the ACE2 pathway leading to direct myocardial injury, but that pathophysiological sequence remains unproven (Hendren 2020). A second hypothesis to explain COVID-19-related myocardial injury centers on cytokine excess and/or antibody mediated mechanisms. It has been also shown that the ACE2 receptor is widely expressed on endothelial cells and that direct SARS-CoV-2 infection of the endothelial cell is possible, leading to diffuse endothelial inflammation (Varga 2020). Post-mortem examination cases indicating a strong virus-induced vascular dysfunction (Menter 2020).

Clinically, COVID-19 can manifest with an acute cardiovascular syndrome (termed “ACovCS”). Numerous cases with ACovCS have been described, not only with typical thoracic complaints, but also with very diverse cardiovascular manifestations. Troponin is an important parameter (see below). In a case series of 18 COVID-19 patients who had ST segment elevation, there was variability in presentation, a high prevalence of nonobstructive disease, and a poor prognosis. 6/9 patients undergoing coronary angiography had obstructive disease. Of note, all 18 patients had elevated D-dimer levels (Bangalore 2020).

In patients with a seemingly typical coronary heart syndrome, COVID-19 should also be considered in the differential diagnosis, even in the absence of fever or cough (Fried 2020, Inciardi 2020). For more information, see the chapter comorbidities.

Thrombosis, embolism

Coagulation abnormalities occur frequently in association with COVID-19, complicating clinical management. Numerous studies have reported on an incredibly high number of venous thromboembolism (VTE), especially in those with severe COVID-19. The initial coagulopathy of COVID-19 presents with prominent elevation of D-dimer and fibrin/fibrinogen degradation products,

while abnormalities in prothrombin time, partial thromboplastin time, and platelet counts are relatively uncommon (excellent review: [Connors 2020](#)). Coagulation test screening, including the measurement of D-dimer and fibrinogen levels, is suggested.

But what are the mechanisms? Some studies have found pulmonary embolism with or without deep venous thrombosis, as well as presence of recent thrombi in prostatic venous plexus, in patients with no history of VTE, suggesting *de novo* coagulopathy in these patients with COVID-19. Others have highlighted changes consistent with thrombosis occurring within the pulmonary arterial circulation, in the absence of apparent embolism (nice review: [Deshpande 2020](#)). Some studies have indicated severe hypercoagulability rather than consumptive coagulopathy ([Spiezia 2020](#)).

Some of the key studies are listed here:

- Of 240 patients (109 critically ill) admitted to US hospitals, VTE was diagnosed in 31 patients (28%) 8 ± 7 days after admission. The authors conclude that routine chemical VTE prophylaxis may be inadequate ([Maatman 2020](#)).
- In a single-center study from Amsterdam on 198 hospitalized cases, the cumulative incidences of VTE at 7 and 21 days were 16% and 42%. In 74 ICU patients, cumulative incidence was 59% at 21 days, despite thrombosis prophylaxis. Authors recommend performing screening compression ultrasound in the ICU every 5 days ([Middeldorp 2020](#)).
- Of 143 patients hospitalized with COVID-19, 66 patients developed lower extremity deep venous thrombosis (46%), among them 23 with proximal DVT ([Zhang L 2020](#)). Patients with DVT were older and had a lower oxygenation index, a higher rate of cardiac injury, and worse prognosis. Multivariate analysis found CURB-65 score 3-5 (OR 6.1), Padua predic-

tion score ≥ 4 (OR 4.0), and D-dimer $>1.0 \mu\text{g/ml}$ (OR 5.8) to be associated with DVT.

- Among the first 107 COVID-19 patients admitted to the ICU for pneumonia in Lille, France, the authors identified 22 (21%) cases of pulmonary embolism (PE). At the time of diagnosis, 20/22 were receiving prophylactic antithrombotic treatment (UFH or LWMH) according to the current guidelines in critically ill patients.
- In 100 patients with severe COVID-19, a high prevalence of 23% was found for pulmonary embolus (PE) ([Grillet 2020](#)). PE was diagnosed at a mean of 12 days from symptom onset. In multivariable analysis, requirement for mechanical ventilation remained associated with acute pulmonary embolus.
- In a prospective study from France, 64/150 (43%) patients were diagnosed with clinically relevant thrombotic complications. The authors argue for higher anticoagulation targets in critically ill patients ([Helms 2020](#)).
- Autopsy findings from 12 patients, showing that 7/12 had deep vein thrombosis. Pulmonary embolism was the direct cause of death in four cases ([Wichmann 2020](#)).
- Careful examination of the lungs from deceased COVID-19 patients with lungs from 7 patients who died from ARDS secondary to influenza A showed distinctive vascular features. COVID-19 lungs displayed severe endothelial injury associated with the presence of intracellular virus and disrupted cell membranes. Histologic analysis of pulmonary vessels showed widespread thrombosis with microangiopathy. Alveolar capillary microthrombi and the amount of vessel growth were 9 and almost 3 times as prevalent as in influenza, respectively ([Ackermann 2020](#)).
- Five cases of large-vessel stroke occurring in younger patients (age 33-49, 2 without any risk factors) ([Oxley 2020](#)).

- Five cases with profound hemodynamic instability due to the development of acute cor pulmonale, among them 4 younger than 65 years of age (Creel-Bulos 2020).

There is a very controversial debate about a possible correlation between the use of ibuprofen and the increased risk of VTE development. According to a recent review (Arjomandi 2020), the causation between the effects of ibuprofen and VTE remains speculative. The role of ibuprofen on a vascular level remains unclear as well as whether ibuprofen is able to interact with SARS-CoV-2 mechanistically. However, the authors recommend careful considerations on avoiding high dosage of ibuprofen in subjects at particular risk of thromboembolic events.

Neurologic symptoms

Neuroinvasive propensity has been demonstrated as a common feature of human coronaviruses. Viral neuroinvasion may be achieved by several routes, including trans-synaptic transfer across infected neurons, entry via the olfactory nerve, infection of vascular endothelium, or leukocyte migration across the blood-brain barrier (review: Zubair 2020). With regard to SARS-CoV-2, early occurrences such as olfactory symptoms (see above) should be further evaluated for CNS involvement. Potential late neurological complications in cured COVID-19 patients are possible (Baig 2020). A retrospective, observational case series found 78/214 patients (36%) with neurologic manifestations, ranging from fairly specific symptoms (loss of sense of smell or taste, myopathy, and stroke) to more non-specific symptoms (headache, low consciousness, dizziness, or seizure). Whether these more non-specific symptoms are manifestations of the disease itself remains to be seen (Mao 2020).

There are several observational series of specific neurological features such as Guillain-Barré syndrome (Toscano 2020) or Mil-

ler Fisher Syndrome and polyneuritis cranialis ([Gutierrez-Ortiz 2020](#)).

Especially in patients with severe COVID-19, neurological symptoms are common. In an observational series of 58 patients, ARDS due to SARS-CoV-2 infection was associated with encephalopathy, prominent agitation and confusion, and corticospinal tract signs. Patients with COVID-19 might experience delirium, confusion, agitation, and altered consciousness, as well as symptoms of depression, anxiety, and insomnia (review: [Rogers 2020](#)). It remains unclear which of these features are due to critical illness-related encephalopathy, cytokines, or the effect or withdrawal of medication, and which features are specific to SARS-CoV-2 infection ([Helms 2020](#)).

Dermatological symptoms

Numerous studies have reported on cutaneous manifestations seen in the context of COVID-19. The most prominent phenomenon, the so-called “COVID toes”, are chilblain-like lesions which mainly occur at acral areas. These lesions can be painful (sometimes itchy, sometimes asymptomatic) and may represent the only symptom or late manifestations of SARS-CoV-2 infection. Of note, in most patients with “COVID toes”, the disease is only mild to moderate. It is speculated that the lesions are caused by inflammation in the walls of blood vessels, or by small micro-clots in the blood. However, whether “COVID toes” represent a coagulation disorder or a hypersensitivity reaction is not yet known. In addition, in many patients, SARS-CoV-2 PCR was negative (or not done) and serology testings (to prove the relationship) are still pending. Key studies:

- Two different patterns of acute acro-ischemic lesions can overlap ([Fernandez-Nieto 2020](#)). The chilblain-like pattern was present in 95 patients (72.0%). It is characterized by red to violet macules, plaques and nodules, usually at the distal

aspects of toes and fingers. The erythema multiform-like pattern was present in 37 patients (28.0%).

- Five clinical cutaneous of lesions are described ([Galvan 2020](#)): acral areas of erythema with vesicles or pustules (pseudo-chilblain) (19%), other vesicular eruptions (9%), urticarial lesions (19%), maculopapular eruptions (47%) and livedo or necrosis (6%). Vesicular eruptions appear early in the course of the disease (15% before other symptoms). The pseudo-chilblain pattern frequently appears late in the evolution of the COVID-19 disease (59% after other symptoms).
- In a case series on 22 adult patients with varicella-like lesions ([Marzano 2020](#)), typical features were constant trunk involvement, usually scattered distribution and mild/absent pruritus, the latter being in line with most viral exanthems but not like true varicella. Lesions generally appeared 3 days after systemic symptoms and disappeared by day 8.
- Three cases of COVID-19 associated ulcers in the oral cavity, with pain, desquamative gingivitis, and blisters ([Martin Carreras-Presas 2020](#)).

Other case reports include digitate papulosquamous eruption ([Sanchez 2020](#)), petechial skin rash ([Díaz-Guimaraens 2020](#), [Quintana-Castanedo 2020](#)). However, it should be kept in mind that not all rashes or cutaneous manifestations seen in patients with COVID-19 can be attributed to the virus. Coinfections or medical complications have to be considered. Comprehensive mucocutaneous examinations, analysis of other systemic clinical features or host characteristics, and histopathologic correlation, will be vital to understanding the pathophysiologic mechanisms of what we are seeing on the skin (Review: [Madigan 2020](#)).

Kidneys and liver

SARS-CoV-2 has an organotropism beyond the respiratory tract, including the kidneys and the liver. Researchers have quantified the SARS-CoV-2 viral load in precisely defined kidney compartments obtained with the use of tissue microdissection from 6 patients who underwent autopsy (Puelles 2020). Three of these 6 patients had a detectable SARS-CoV-2 viral load in all kidney compartments examined, with preferential targeting of glomerular cells. Renal tropism is a potential explanation of commonly reported new clinical signs of kidney injury in patients with COVID-19, even in patients with SARS-CoV-2 infection who are not critically ill (Zhou 2020). Recent data indicate that renal involvement is more frequent than described in early studies. Of the first 1,000 patients presenting at the NewYork-Presbyterian-Columbia University, 236 were admitted or transferred to intensive care units (Argenziano 2019). Of these, 78.0% (184/236) developed acute kidney injury and 35.2% (83/236) needed dialysis. Concomitantly, 13.8% of all patients and 35.2% of patients in intensive care units required in-patient dialysis, leading to a shortage of equipment needed for dialysis and continuous renal replacement therapy.

One of the largest studies, evaluating liver injury in 2,273 SARS-CoV-2 positive patients, found that 45% had mild, 21% moderate, and 6.4% severe liver injury. In multivariable analysis, severe acute liver injury was significantly associated with elevated inflammatory markers including ferritin and IL-6. Peak ALT was significantly associated with death or discharge to hospice (OR 1.14, $p=0.044$), controlling for age, body mass index, diabetes, hypertension, intubation, and renal replacement therapy (Phipps 2020).

Ocular and atypical manifestations

Ocular manifestations are also common. In a case series from China, 12/38 patients (32%, more common in severe cases) had ocular manifestations consistent with conjunctivitis, including conjunctival hyperemia, chemosis, epiphora, or increased secretions. Two patients had positive PCR results from conjunctival swabs (Wu 2020). The retina can also be affected, as has been shown using optical coherence tomography (OCT), a non-invasive imaging technique that is useful for demonstrating sub-clinical retinal changes. Twelve adult patients showed hyper-reflective lesions at the level of the ganglion cell and inner plexiform layers more prominently at the papillomacular bundle in both eyes (Marinho 2020).

Other new and sometimes puzzling clinical presentations have emerged (and will emerge) in the current pandemic. There are case reports of non-specific symptoms, especially in the elderly population, underlining the need for extensive testing in the current pandemic (Nickel 2020).

Laboratory findings

The most evident laboratory findings in the first large cohort study from China (Guan 2020) are shown in Table 1. On admission, lymphocytopenia was present in 83.2% of the patients, thrombocytopenia in 36.2%, and leukopenia in 33.7%. In most patients, C-reactive protein was elevated to moderate levels; less common were elevated levels of alanine aminotransferase, and D-dimer. Most patients have normal procalcitonin on admission.

Table 2. Percentage of symptoms in first larger cohort study from China (Guan 2020). Disease severity was classified according to American Thoracic Society (Metlay 2019) guidelines

Clinical symptoms	All	Severe Disease	Non-Severe
Fever,%	88.7	91.9	88.1
Cough,%	67.8	70.5	67.3
Fatigue,%	38.1	39.9	37.8
Sputum production,%	33.7	35.3	33.4
Shortness of breath,%	18.7	37.6	15.1
Myalgia or arthralgia,%	14.9	17.3	14.5
Sore throat,%	13.9	13.3	14.0
Headache,%	13.6	15.0	13.4
Chills,%	11.5	15.0	10.8
Nausea or vomiting,%	5.0	6.9	4.6
Nasal congestion,%	4.8	3.5	5.1
Diarrhea,%	3.8	5.8	3.5
Radiological findings			
Abnormalities on X-ray,%	59.1	76.7	54.2
Abnormalities on CT,%	86.2	94.6	84.4
Laboratory findings			
WBC <4,000 per mm ³ ,%	33.7	61.1	28.1
Lymphocytes <1,500 per mm ³ ,%	83.2	96.1	80.4
Platelets <150,000 per mm ³ ,%	36.2	57.7	31.6
C-reactive protein ≥10 mg/L,%	60.7	81.5	56.4
Lactate dehydrogenase ≥250 U/L,%	41.0	58.1	37.1
AST >40 U/L,%	22.2	39.4	18.2
D-dimer ≥0.5 mg/L,%	46.6	59.6	43.2

Inflammation

Parameters indicating inflammation such as elevated CRP and procalcitonin are very frequent findings. They have been proposed to be important risk factors for disease severity and mortality (Chen 2020). For example in a multivariate analysis of a retrospective cohort of 1,590 hospitalized subjects with COVID-

19 throughout China, a procalcitonin > 0.5 ng/ml at admission had a HR for mortality of 8.7 (95% CI:3.4-22.3). In 359 patients, CRP performed better than other parameters (age, neutrophil count, platelet count) in predicting adverse outcome. Besides, admission serum CRP level was identified as a moderate discriminator of disease severity (Lu 2020). Of 5,279 cases confirmed in a large medical center in New York, 52% of them admitted to hospital, a CRP > 200 was more strongly associated (odds ratio 5.1) with critical illness than age or comorbidities (Petrilli 2019).

In a retrospective observational study of 69 patients with severe COVID-19, the decrease of interleukin-6 (IL-6) levels was closely related to treatment effectiveness, while the increase of IL-6 indicated disease exacerbation. The authors concluded that the dynamic change of IL-6 levels can be used as a marker in disease monitoring in patients with severe COVID-19 (Liu 2020). High levels of IL-6 and IL-8 during treatment were observed in patients with severe or critical disease and correlated with decreased lymphocyte count in another study on 326 patients from China (Zhang 2020). The determinants of disease severity seemed to stem mostly from host factors such as age, lymphocytopenia, and its associated cytokine storm.

Hematological: Lymphocytes, platelets

Lymphocytopenia and transient but severe T cell depletion is a well-known feature of SARS (He 2005). In COVID-19, lymphopenia is also among the most prominent hematological features. Lymphopenia may be predictive for progression (Ji 2020) and patients with severe COVID-19 present with lymphocytopenia of less than $1500/\mu\text{l}$ in almost 100% (Guan 2020). It's not only the total lymphocyte count. There is growing evidence for a transient depletion of T cells. Especially the reduced CD4⁺ and CD8⁺ T cell counts upon admission was predictive of disease progression in a larger study (Zhang 2020). In another large study on COVID-

19 patients, CD3+, CD4+ and CD8+ T cells but also NK cells were significantly decreased in COVID-19 patients and related to the severity of the disease. According to the authors, CD8+ T and CD4+ T cell counts can be used as diagnostic markers of COVID-19 and predictors of disease severity (Jiang 2020).

Another common hematological finding is low platelet counts that may have different causes (Review: Xu 2020). Cases of hemorrhagic manifestation and severe thrombocytopenia responding to immunoglobulins fairly quickly with a sustained response over weeks have been reported (Ahmed 2020).

Cardiac: Troponin

Given the cardiac involvement especially in severe cases (see above), it is not surprising that cardiac parameters are frequently elevated. A meta-analysis of 341 patients found that cardiac troponin I levels are significantly increased only in patients with severe COVID-19 (Lippi 2020). In 179 COVID-19 patients, cardiac troponin ≥ 0.05 ng/mL was predictive for mortality (Du 2020). In a huge cohort study from New York, troponin was strongly associated with critical illness (Petrilli 2019). However, it remains to be seen whether troponin levels can be used as a prognostic factor. A comprehensive review on the interpretation of elevated troponin levels in COVID-19 was recently published (Chapman 2020).

Coagulation: D-Dimer, aPTT

Several studies have evaluated the coagulation parameter D-dimer in the progression of COVID-19. Among 279 patients in whom D-dimer was measured for ten consecutive days after admission, dynamically changes positively correlated with the prognosis (Li 2020). In the Wuhan study, all patients surviving had low D-dimer during hospitalization, whereas levels in non-survivors tended to increase sharply at day 10. In a multivariate

analysis, D-dimer of $> 1 \mu\text{g/mL}$ remained the only lab finding which was significantly associated with in-hospital death, with an odds ratio of 18.4 (2.6-129, $p=0.003$). However, D-dimer has a reported association with mortality in patients with sepsis and many patients died from sepsis (Zhou 2020).

In a considerable proportion of patients, a prolonged aPTT can be found. Of 216 patients with SARS-CoV-2, this was the case in 44 (20%). Of these, 31/34 (91%) had positive lupus anticoagulant assays. As this is not associated with a bleeding tendency, it is recommended that prolonged aPTT should not be a barrier to the use of anticoagulation therapies in the prevention and treatment of venous thrombosis (Bowles 2020). Another case series of 22 patients with acute respiratory failure present a severe hypercoagulability rather than consumptive coagulopathy. Fibrin formation and polymerization may predispose to thrombosis and correlate with a worse outcome (Spiezia 2020).

Lab findings as risk factor

It is not very surprising that patients with severe disease had more prominent laboratory abnormalities than those with non-severe disease. It remains unclear, how a single parameter can be of clinical value as almost all studies were retrospective and uncontrolled. Moreover, the numbers of patients were low in many studies. However, there are some patterns which may be helpful in clinical practice. Lab risk factors are:

- Elevated CRP, procalcitonin, interleukin-6 and ferritin
- Lymphocytopenia, CD4 T cell and CD8 T cell depletion, leukocytosis
- Elevated D-dimer and troponin
- Elevated LDH

Clinical classification

There is no broadly accepted or valid clinical classification for COVID-19. The first larger clinical study distinguished between severe and non-severe cases ([Guan 2020](#)), according to the Diagnosis and Treatment Guidelines for Adults with Community-acquired Pneumonia, published by the American Thoracic Society and Infectious Diseases Society of America ([Metlay 2019](#)). In these validated definitions, severe cases include either one major criterion or three or more minor criteria. Minor criteria are a respiratory rate > 30 breaths/min, $\text{PaO}_2/\text{FI}\text{O}_2$ ratio < 250 , multilobar infiltrates, confusion/disorientation, uremia, leukopenia, low platelet count, hypothermia, hypotension requiring aggressive fluid resuscitation. Major criteria comprise septic shock with need for vasopressors or respiratory failure requiring mechanical ventilation.

Some authors ([Wang 2020](#)) have used the following classification including four categories:

1. Mild cases: clinical symptoms were mild without pneumonia manifestation through image results
2. Ordinary cases: having fever and other respiratory symptoms with pneumonia manifestation through image results
3. Severe cases: meeting any one of the following: respiratory distress, hypoxia ($\text{SpO}_2 \leq 93\%$), abnormal blood gas analysis: ($\text{PaO}_2 < 60\text{mmHg}$, $\text{PaCO}_2 > 50\text{mmHg}$)
4. Critical cases: meeting any one of the following: Respiratory failure which requires mechanical ventilation, shock, accompanied by other organ failure that needs ICU monitoring and treatment.

In the report of the Chinese CDC, estimation of disease severity used almost the same categories ([Wu 2020](#)) although numbers 1 and 2 were combined. According to the report, there were 81% mild and moderate cases, 14% severe cases and 5% critical cases.

There are preliminary reports from the Italian National Institute of Health, reporting on 24.9% severe and 5.0% critical cases ([Livingston 2020](#)). However, these numbers are believed to strongly overestimate the disease burden, given the very low number of diagnosed cases in Italy at the time. Among 7,483 US health care workers with COVID-19, a total of 184 (2.1–4.9%) had to be admitted to ICUs. Rate was markedly higher in HCWs older 65 of age, reaching 6.9–16.0% ([CDC 2020](#)).

Outcome

We are facing rapidly increasing numbers of severe and fatal cases in the current pandemic. The two most difficult but most frequently asked clinical questions are 1. How many patients end up with severe or even fatal courses of COVID-19? 2. What is the true proportion of asymptomatic infections? We will learn more about this shortly through serological testing studies. However, it will be important that these studies are carefully designed and carried out, especially to avoid bias and confounding.

Case fatality rates

The case fatality rates (CFR) or infection fatality rates (IFR) are both difficult to assess in such a dynamic pandemic. CFR can be biased upwards by underreporting of cases and downwards by insufficient follow up or unknown outcome. A downward trend may also indicate improvements in epidemiological surveillance. COVID-19 fatality is likely overestimated and especially early estimates are susceptible to uncertainty about asymptomatic or subclinical infections and several biases, including biases in detection, selection or reporting ([Niforatos 2020](#)).

Dividing the number of deaths by the number of total confirmed cases is not appropriate. For example, on May 30, the CFR between the 30 most affected countries (in terms of absolute numbers) ranged from 0.07 (Singapore) to 16.7 (Belgium). Within the

10 most affected countries, the range was 1.15 (Russia) to 15.3 (France).

The picture is much more complex and these simple calculations certainly do not reflect the true mortality in each country without taking into factor three other issues:

1. The testing policies (and capacities) in a country. This is the most important factor. The fewer people you test (all people, only symptomatic patients, only those with severe symptoms) the higher the mortality. In Germany, testing systems and high lab capacities were established rapidly ([Stafford 2020](#)).
2. Age of the infected population and especially of the population which is affected first. For example, in Italy, higher percentages of older people became infected during the first weeks, compared to Germany (where many people acquired SARS-CoV during ski holidays or carnival sessions). Especially if high-risk sites (such as retirement homes) are affected, death cases in the country will increase considerably. For example, a single outbreak in Washington has led to 34 deaths among 101 residents of a long-term care facility ([McMichael 2020](#)) – this is exactly the same number of death cases which Australia has reported as whole country on April 4, among a total of 5,635 confirmed COVID-19 cases.
3. Stage of the epidemic. Some countries have experienced their epidemic grow early, some are still a few days or weeks behind. Death rates only reflect the infection rate of 2-3 weeks previously. In the large retrospective study from Wuhan, the time from illness onset to death was 18.5 days (IQR 15-22 days).

The “death rates” for some selected countries, based on the number of deaths and tests, is shown in Figure 1. These curves reflect test readiness and test capacities. A country such as Sweden, which initially relied on “herd immunity”, differs signifi-

cantly from countries in which a lot has been tested from the beginning of the epidemic, such as Germany. The USA is still at the beginning, in Korea the outbreak was stopped relatively quickly by intensive tracking measures.

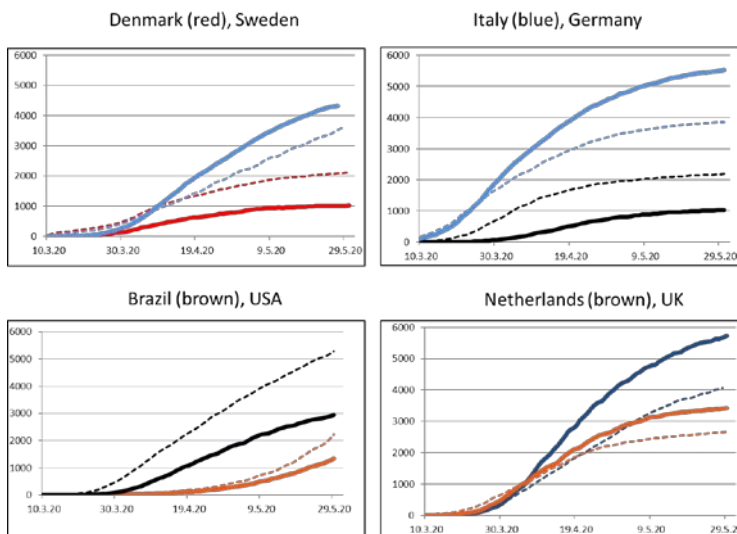


Figure 1. People who tested positive (among 1 million inhabitants, dashed) and deaths (among 10 million inhabitants). "Mortality" reaches 10% at the point where the curves intersect. This has happened for countries such as UK, Italy or Sweden, but is unlikely for others like Germany, Switzerland or USA.

CFR among health care workers and among well-defined populations

In well-monitored populations in which underreporting is unlikely or can be largely determined, the mortality rates may better reflect the "true" CFR of COVID-19. This applies to healthcare workers (HCW) but also to populations of "well-defined" (lim-

ited) outbreaks. The low mortality rates in these populations are remarkable.

In large study of 3,387 HCW from China infected with SARS-CoV-2, only 23 have died, corresponding to a mortality of 0.68%. The median age was 55 years (range, 29 to 72), and 11 of the 23 deceased HCW had been reactivated from retirement (Zhan 2020). Current studies in the USA have found similar rates, mortality estimates were 0.3-0.6% (CDC 2020). Of the 27 HCW who have died from COVID-19 until mid-April, 18 were over 54 years of age. The overall low mortality rates were probably due to the fact that HCWs were younger and healthier, but also that they had been tested earlier and more frequently.

We will also learn more from limited outbreaks affecting homogeneous populations, such as cruise ships and aircraft carriers. Outbreaks on these floating microcosms are unfortunate but informative experiments, they tell us a lot about transmission and the natural course of the disease in well-defined populations. Two large “involuntary field studies” are currently taking place: around 1,140 sailors were infected on the US aircraft carrier Theodore Roosevelt (one soldier has already died, nine were hospitalized), and more than 1,080 COVID-19 patients on the French aircraft carrier Charles de Gaulle. These populations are probably young, healthy and correspond more to the general population. Detailed investigations will follow.

The most valid data seem to come from the Diamond Princess. As of May 31, the total number of infected reached 712, and 13 patients have died from the disease leading to a CFR of 1.8%. However, this rate may yet increase, as at least 4 patients were in serious condition (Moriarty 2020). Of note, around 75% of the patients on the Diamond Princess were of 60 years or older, many of them in their eighties. Projecting the Diamond Princess case fatality rate onto the age structure of the general population, it is obvious that the mortality rate may be much lower in

other broader populations. Mortality would be in a range of 0.2-0.4 %.

Older Age

From the beginning of the epidemic, older age has been identified as an important risk factor for disease severity (Huang 2020, Guan 2020). In Wuhan, there was a clear and considerable age dependency in symptomatic infections (susceptibility) and outcome (fatality) risks, by multiple folds in each case (Wu 2020). The summarizing report from the Chinese CDC found a death rate of 2.3%, representing 1,023 among 44,672 confirmed cases (Wu 2020). Mortality increased markedly in older people. In the cases aged 70 to 79 years, CFR was 8.0% and cases in those aged 80 years older had a 14.8% CFR.

In recent weeks, this has been seen and confirmed by almost all studies published throughout the world. In almost all countries, age groups of 80 years of older contribute to more than 90% of all death cases.

- In a large registry analysing the epidemic in the UK in 20,133 patients, the median age of the 5,165 patients (26%) who died in hospital from COVID-19 was 80 years (Docherty 2020).
- Among 1,591 patients admitted to ICU in Lombardy, Italy, older patients (> 63 years) had markedly higher mortality than younger patients (36% vs 15%). Of 362 patients older than 70 years of age, mortality was 41% (Grasselli 2020).
- According to the Italian National Institute of Health, an analysis of the first 2,003 death cases, median age was 80.5 years. Only 17 (0.8%) were 49 years or younger, and 88% were older than 70 years (Livingston 2020).
- Detailed analysis of all-cause mortality at Italian hot spots showed that the deviation in all-cause deaths compared to previous years during epidemic peaks was largely driven by

the increase in deaths among older people, especially in men (Piccininni 2020, Michelozzi 2020).

- In 5,700 patients admitted to New York hospitals, there was a dramatic increase of mortality among older age groups, reaching 61% (122/199) in men and 48% (115/242) in women over 80 years of age (Richardson 2020).
- In an outbreak reported from King County, Washington, a total of 167 confirmed cases were observed in 101 residents (median age 83 years) of a long-term care facility, in 50 healthcare workers (HCW, median age 43 years), and 16 visitors. The case fatality rate for residents was 33.7% (34/101) and 0% among HCW (McMichael 2020).

There is no doubt that older age is by far the most important risk factor for mortality. Countries failing to protect their elderly population for different reasons (such as Italy, Belgium or Sweden) are facing higher CFR, while those without many older patients infected by SARS-CoV-2 (such as the Republic of Korea, Singapore, Australia) have markedly lower rates.

Other risk factors for severe disease

Besides older age, many risk factors for severe disease and mortality have been evaluated in the current pandemic. Early studies from China found comorbidities such as hypertension, cardiovascular disease and diabetes to be associated with severe disease and death (Guan 2020). Among 1,590 hospitalised patients from mainland China, after adjusting for age and smoking status, COPD (hazard ratio, 2.7), diabetes (1.6), hypertension (1.6) and malignancy (3.5) were risk factors of reaching clinical endpoints (Guan 2020). Dozens of further studies have also addressed risk factors (Shi 2020, Zhou 2020). The risk scores that have been mainly proposed by Chinese researchers are so numerous that

they cannot be discussed here. They were mainly derived from uncontrolled data, their clinical relevance remains limited.

During recent weeks, several studies conducted outside China have found obesity to be an important risk factor (Goyal 2020, Petrilli 2019). Among the first 393 consecutive patients who were admitted to two hospitals in New York City, obese patients were more likely to require mechanical ventilation. Obesity was also an important risk factor in France (Caussy 2020) or in Singapore, especially in younger patients (Ong 2020). Smoking as a risk factor is under discussion, as well as COPD, kidney diseases and many others (see comorbidity chapter). Among 1,150 adults who were admitted to two NYC hospitals with COVID-19 in March, older age, chronic cardiac disease (adjusted HR 1.76) and chronic pulmonary disease (2.94) were independently associated with in-hospital mortality (Cummings 2020).

The hitherto largest registry data from different parts of the world are shown in Table 3. A striking finding of these studies is the lower mortality in female patients, running through almost all available data. There is some evidence that there are sex-specific differences in clinical characteristics and prognosis and that the presence of comorbidities is of less impact in females (Meng 2020). It has been speculated that the higher vulnerability in men is due to the presence of subclinical systemic inflammation, blunted immune system, down-regulation of ACE2 and accelerated biological aging (Bonafè 2020).

The main problem of all studies published to date is that their uncontrolled data is subject to confounding and that they do not prove causality. Even more importantly: The larger the numbers, the more imprecise the definition of a given comorbidity. What is a “chronic cardiac disease”? A mild and well-controlled hypertension or a severe cardiomyopathy? The clinical manifestation and the relevance of a certain comorbidity may be very heterogeneous (see also the comorbidity chapter).

There is growing evidence that sociodemographic factors play a role. Many studies did not adjust for these factors. For example, in a large cohort of 3,481 patients in Louisiana, public insurance (Medicare or Medicaid), residence in a low-income area, and obesity were associated with increased odds of hospital admission ([Price-Haywood 2020](#)). A careful investigation of the NYC epidemic revealed that the Bronx, which has the highest proportion of racial/ethnic minorities, the most persons living in poverty, and the lowest levels of educational attainment, had higher rates (almost two-fold) of hospitalization and death related to COVID-19 than the other 4 NYC boroughs Brooklyn, Manhattan, Queens and Staten Island ([Wadhera 2020](#)).

Taken together, large registry studies have found slightly elevated Hazard Ratios of mortality for multiple comorbidities (Table 3). It seems, however, that most patients with preexisting conditions are able to control and eradicate the virus. Comorbidities play a major role in those who do not resolve and who fail to limit the disease to an upper respiratory tract infection and who develop pneumonia. Facing the devastation that COVID-19 can inflict not only on the lungs but on many organs, including blood vessels, heart and kidneys (nice review: [Wadman 2020](#)), it seems plausible that a decreased cardiovascular and pulmonary capacity ameliorate clinical outcome in these patients.

However, at this time, we can only speculate about the precise role of comorbidities and their mechanisms to contribute to disease severity.

Is there a higher susceptibility? In a large, population-based study from Italy, patients with COVID-19 had a higher baseline prevalence of cardiovascular conditions and diseases (hypertension, coronary heart disease, heart failure, and chronic kidney disease). The incidence was also increased in patients with previous hospitalizations for cardiovascular or noncardiovascular diseases ([Mancia 2020](#)). A large UK study found some evidence of

potential sociodemographic factors associated with a positive test, including deprivation, population density, ethnicity, and chronic kidney disease (Lusignan 2020). However, even these well performed studies cannot completely rule out the (probably strong) diagnostic suspicion bias. Patients with comorbidities could be more likely to present for assessment and be selected for SARS-CoV-2 testing in accordance with guidelines. Given the high number of nosocomial outbreaks, they may also at higher risk for infection, just due to higher hospitalization rates.

Table 3. Age and comorbidities in a large registry study (Docherty 2020), providing multivariate analyses and Hazard Ratios.

	UK, n = 15,194
Hazard Ratio (95% CI)	Death
Age 50-59 vs < 50	2.63 (2.06-3.35)
Age 60-69 vs < 50	4.99 (3.99-6.25)
Age 70-79 vs < 50	8.51 (6.85-10.57)
Age > 80 vs < 50	11.09 (8.93-13.77)
Female	0.81 (0.75-0.86)
Chronic cardiac disease	1.16 (1.08-1.24)
Chronic pulmonary disease	1.17 (1.09-1.27)
Chronic kidney disease	1.28 (1.18-1.39)
Hypertension	
Diabetes	1.06 (0.99-1.14)
Obesity	1.33 (1.19-1.49)
Chronic neurological disorder	1.18 (1.06-1.29)
Dementia	1.40 (1.28-1.52)
Malignancy	1.13 (1.02-1.24)
Moderate/severe liver disease	1.51 (1.21-1.88)

Predisposition

COVID-19 shows an extremely variable course, from completely asymptomatic to fulminantly fatal. In some cases it affects young and apparently healthy people, for whom the severity of the disease is neither caused by age nor by any comorbidities – just think of the Chinese doctor Li Wenliang, who died at the age of 34 from COVID-19 (see chapter Timeline). So far, only assumptions can be made. The remarkable heterogeneity of disease patterns from a clinical, radiological, and histopathological point of view has led to the speculation that the idiosyncratic responses of individual patients may be in part related to underlying genetic variations ([von der Thusen 2020](#)). Some preliminary reports suggest that this is the case.

- For example, a report from Iran describes three brothers aged 54 to 66 who all died of COVID-19 after less than two weeks of fulminating progress. All three had previously been healthy, without underlying illnesses ([Yousefzadegan 2020](#)).
- In a post-mortem examination of 21 COVID-19 cases, 65% of the deceased patients had blood group A. Blood group A may be associated with the failure of pulmonary microcirculation and coagulopathies. Another explanation could be the direct interaction between antigen A and the viral S protein, thus facilitating virus entry via ACE2 ([Menter 2020](#)).
- Researchers from UK have investigated the associations between ApoEε4 alleles and COVID-19 severity, using the UK Biobank data ([Kuo 2020](#)). ApoEε4ε4 homozygotes were more likely to be COVID-19 test positives (odds Ratio 2.31, 95% CI: 1.65-3.24) compared to ε3ε3 homozygotes. The ApoEε4ε4 allele increased risks of severe COVID-19 infection, independent of pre-existing dementia, cardiovascular disease, and type 2 diabetes. This interesting observation needs to be confirmed (and explained).

In addition to the genetic predisposition, other potential reasons for a severe course need to be considered: the amount of viral exposure (probably high for Li Wenliang?), the route by which the virus enters the body, ultimately also the virulence of the pathogen and a possible (partial) immunity from previous viral diseases. If you inhale large numbers of virus deeply, leading rapidly to a high number of virus in the pulmonary system, this may be much worse than smearing a small amount of virus on your hand to the nose. In this case, the immune system in the upper respiratory tract may have much more time to limit further spread into the lungs and other organs. But this is still speculation and will have to be investigated in the coming months.

Overburdened health care systems

Mortality may be also higher in situations where hospitals are unable to provide intensive care to all the patients who need it, in particular ventilator support. Mortality would thus also be correlated with health-care burden. Preliminary data show clear disparities in mortality rates between Wuhan (>3%), different regions of Hubei (about 2.9% on average), and across the other provinces of China (about 0.7% on average). The authors have postulated that this is likely to be related to the rapid escalation in the number of infections around the epicenter of the outbreak, which has resulted in an insufficiency of health-care resources, thereby negatively affecting patient outcomes in Hubei, while this has not yet been the situation in other parts of China (Ji 2020). Another study estimated the risk of death in Wuhan as high as 12% in the epicentre and around 1% in other more mildly affected areas (Mizumoto 2020).

The nightmare of insufficient resources is currently the reality in Northern Italy. In Italy, on March 15, the cumulative death numbers exceeded for the first time those of admissions to in-

tensive care units – a clear sign for a collapsing health care system. Other countries or regions will face the same situation soon.

Reactivations, reinfections

There are several reports of patients who become positive again after negative PCR tests (Lan 2020, Xiao 2020, Yuan 2020). These reports have gained much attention, because this could indicate both reactivations as well as reinfections. After closer inspection of these reports, however, there is no good evidence for reactivations or reinfections, and other reasons are much more likely. Methodological problems of PCR always have to be considered; the results can considerably fluctuate (Li 2020). Insufficient material collection or storage are just two examples of many problems with PCR. Even if everything is done correctly, it can be expected that a PCR could fluctuate between positive and negative at times when the values are low and the viral load drops at the end of an infection (Wölfel 2020). It also depends on the assay used, the detection limit is between a few hundred and several thousand virus copies/mL (Wang 2020).

The largest study to date found a total of 25 (14.5%) of 172 discharged COVID-19 patients who had a positive test at home after two negative PCR results at hospital (Yuan 2020). On average, the time between the last negative and the first positive test was 7.3 (standard deviation 3.9) days. There were no differences to patients who remained negative. This and the short period of time suggest that in these patients, no reactivations are to be expected.

In addition, animal studies suggest that re-infection is very unlikely (Chandrashekar 2020). Following initial viral clearance and on day 35 following initial viral infection, 9 rhesus macaques were re-challenged with the same doses of virus that were utilized for the primary infection. Very limited viral RNA was observed in BAL on day 1, with no viral RNA detected at subsequent

timepoints. These data show that SARS-CoV-2 infection induced protective immunity against re-exposure in nonhuman primates. Reactivations as well as rapid new infections would be very unusual, especially for coronaviruses. If a lot of testing is done, you will find a number of such patients who become positive again after repeated negative PCR and clinical convalescence. The phenomenon is likely to be overrated. Most patients get well anyway; moreover, it is unclear whether renewed positivity in PCR is synonymous with infectiousness.

Outlook

Over the coming months, serological studies will give a clearer picture of the true number of asymptomatic patients and those with unusual symptoms. More importantly, we have to learn more about risk factors for severe disease, in order to adapt prevention strategies. Older age is the main but not the only risk factor. Recently, a 106-year-old COVID-19 patient recently recovered in the UK. The precise mechanisms how comorbidities (and comedications) may contribute to an increased risk for a severe disease course have to be elucidated. Genetic and immunological studies have to reveal susceptibility and predisposition for both severe and mild courses. Who is really at risk, who is not? Quarantining only the old is too easy.

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8. Treatment

Christian Hoffmann

The number of people infected with SARS-CoV-2 is increasing rapidly. Because up to 5-10% can have a severe, potentially life-threatening course, there is an urgent need for effective drugs. No proven effective therapy for this virus currently exists. The time in this pandemic is too short for the development of new, specific agents; a vaccine will also be a long time coming. Thus, existing antivirals or immune modulators with known safety profiles will gain traction as the fastest route to fight COVID-19. Those compounds that have already been tested in other indications now have priority, in particular those that have been shown to be effective in other beta-coronaviruses such as SARS and MERS.

Many current suggestions have emerged from animal models, cell lines or even virtual screening models. While some approaches have at least some evidence for clinical benefit, for others this remains highly speculative. A brief look at ClinicalTrials.gov may illustrate the intensive research efforts that are underway: on April 18, the platform listed 657 studies, with 284 recruiting, among them 121 in Phase III randomized clinical trials (RCTs, assessed on April 19). On May 31, these numbers have increased to 1844, 926 and 126.

Several very different therapeutic approaches are in the treatment pipeline for COVID-19: antiviral compounds that inhibit enzyme systems, those inhibiting the entry of SARS-CoV-2 into the cell and, finally, immunomodulators that are supposed to reduce the cytokine storm and associated pulmonary damage that is seen in severe case. In an interim guidance, the WHO stated on March 13, that “there is no current evidence to recommend any specific anti-COVID-19 treatment” and that use of in-

vestigational therapeutics “should be done under ethically approved, randomized, controlled trials”. Of note, this has not changed during recent weeks. There is no agent that shows a decreased mortality.

However, performing clinical trials remains challenging during a public health crisis (Rome 2020) and enrolling patients in clinical trials will not be possible everywhere. For these, this chapter may support in decision-making. The following agents will be discussed here:

1. Inhibitors of viral RNA synthesis

RdRp Inhibitors	Remdesivir, Favipiravir (and Ribavirin, Sofosbuvir)
Protease Inhibitors	Lopinavir/r

2. Antiviral Entry Inhibitors

TMPRSS2 Inhibitors	Camostat
Fusion Inhibitors	Umifenovir
Others	Hydroxy/chloroquine, Oseltamivir, Baricitinib

3. Immunomodulators and other immune therapies

Corticosteroids	
IL-6 targeting therapies	Tocilizumab, Siltuximab
Immune modulation	Interferon, Anakinra
Passive immunization	Convalescent plasma, monoclonal antibodies

Inhibitors of the viral RNA synthesis

SARS-CoV-2 is a single-stranded RNA beta-coronavirus. Potential targets are some non-structural proteins such as protease, RNA-dependent RNA polymerase (RdRp) and helicase, but also acces-

sory proteins. Coronaviruses do not use reverse transcriptase. There is only a total of 82% genetic identity between SARS-CoV and SARS-CoV-2. However, the strikingly high genetic homology for one of the key enzymes, the RdRp which reaches around 96% (Morse 2020), suggests that substances effective for SARS may also be effective for COVID-19.

RdRp inhibitors

Remdesivir

Remdesivir (RDV) is a nucleotide analogue and the prodrug of an adenosine C nucleoside which incorporates into nascent viral RNA chains, resulting in premature termination. From WHO, remdesivir has been ranked as the most promising candidate for the treatment of COVID-19. *In vitro* experiments have shown that remdesivir has a broad anti-CoV activity by inhibiting RdRp in airway epithelial cell cultures, even at submicromolar concentrations (Sheahan 2017). This RdRp inhibition also applies to SARS-CoV-2 (Wang 2020). The substance is very similar to tenofovir alafenamide, another nucleotide analogue used in HIV therapy. Remdesivir was originally developed by Gilead Sciences for the treatment of the Ebola virus but was subsequently abandoned, after disappointing results in a large randomized clinical trial (Mulangu 2019). Experimental data from mouse models showed better prophylactic and therapeutic efficacy in MERS than a combination of lopinavir/ritonavir (see below) and interferon beta. Remdesivir improved lung function and reduced viral load and pulmonary damage (Sheahan 2020). Resistance to remdesivir in SARS was generated in cell cultures, but was difficult to select and seemingly impaired viral fitness and virulence (Agostini 2018). The same is seen with MERS viruses (Cockrell 2016). Animal models suggest that a once-daily infusion of 10 mg/kg remdesivir may be sufficient for treatment; pharmacokinetic data for humans are still lacking. Gilead is currently “in the

process” of opening expanded access programs in Europe (refer to [gilead.com](https://www.gilead.com)). In the US, this program is already in place.

Clinical data: Safety was shown in the Ebola trial. Remdesivir is currently being tested in several RCTs in > 1,000 patients with both mild-to-moderate and with severe COVID-19 disease. Remdesivir is also among four treatment options being tested in the large WHO SOLIDARITY RCT (see below). In the Phase III studies on COVID-19, an initial dose of 200 mg is started on day 1, similar to the Ebola studies, followed by 100 mg for another 4-9 days. The key trials are listed here:

- **Compassionate Use Program:** this was a fragmentary case series ([Grein 2020](#)) on some patients (only 53/61 patients were analyzed) with various disease severity. Some improved, some didn't: random noise. We believe, for a number of reasons, that this case series published in the New England Journal of Medicine is a cautionary tale for “science in a hurry”, arousing false expectations. It might have been preferable to postpone the publication.
- **NCT04257656:** This multicentre trial was conducted between Feb 6 and March 12 at ten hospitals in Hubei ([Wang 2020](#)). A total of 237 patients with pneumonia, oxygen saturation of 94% or lower on room air and within 12 days of symptom onset were randomized to receive 10 days of single infusions or placebo. Clinical improvement was defined as the number of days to the point of a decline of two levels on a six-point clinical scale (from 1 = discharged to 6 = death) or discharged alive from hospital, whichever came first. Patients were 65 years old (IQR 56–71), more male (56%) and many were co-treated with lopinavir (28%) and corticosteroids. The trial did not attain the predetermined sample size because the outbreak was brought under control in China. However, remdesivir was not associated with a difference in time to clinical improvement (hazard ratio 1.23, 95% CI 0.87–1.75).

Clinical improvement rates were 27% versus 23% at day 14 and 65% versus 58% at day 28. Day 28 mortality was 14% versus 13%. Of note, the viral load decreased similarly in both groups. Some patients with remdesivir had dosing prematurely stopped due to adverse events (12% versus 5%, mainly gastrointestinal symptoms and increases of liver enzymes). The positive message from this trial is that time to recovery was “numerically” shorter in the remdesivir group than the control group, particularly in those treated within 10 days of symptom onset.

- **SIMPLE 1:** in this randomized, open-label, Phase III trial in 397 hospitalized patients with severe COVID-19 and not requiring IMV, clinical improvement at day 14 was 64% with 5 days remdesivir and 54% with 10 days ([Goldman 2020](#)). After adjustment for (significant) baseline imbalances in disease severity, outcomes were similar. The most common adverse events were nausea (9%), worsening respiratory failure (8%), elevated ALT level (7%), and constipation (7%). Because the trial lacked a placebo control, it was not a test of efficacy for remdesivir. An expansion phase will enroll an additional 5,600 (!) patients around the world.
- **ACTT (Adaptive COVID-19 Treatment Trial):** The conclusion of this double-blinded Phase III study that randomized 1,063 COVID-19 patients throughout the world to the drug or to placebo, was remarkably short: “Remdesivir was superior to placebo in shortening the time to recovery in adults hospitalized with Covid-19 and evidence of lower respiratory tract infection” ([Beigel 2020](#)). Median recovery time was 11 versus 15 days. The benefit was most apparent in patients with a baseline ordinal score of 5 (requiring oxygen but not high-flow oxygen). In patients requiring mechanical ventilation or ECMO, there was no effect at all (although the numbers were low). Gender, ethnicity, age or symptom duration had no im-

pact. The Kaplan-Meier estimates of mortality by 14 days were 7.1% and somewhat (not significantly) lower with remdesivir compared to 11.9% with placebo (hazard ratio for death, 0.70; 95% CI, 0.47 to 1.04). These results are preliminary. The full analysis of the entire trial population is expected to be published soon.

What comes next? Several additional trials are ongoing. Some have been suspended such as NCT04252664, a trial in adults with mild and moderate COVID-19, as during the last few weeks no eligible patients could be recruited. The second SIMPLE trial, NCT04292730 (GS-US-540-5774) is probably the most interesting study, evaluating the efficacy of two remdesivir regimens compared to standard of care in 600 patients with moderate COVID-19, with respect to clinical status assessed by a 7-point ordinal scale on day 11. Estimated study completion date is May 2020. INSERM in France has initiated a study evaluating remdesivir and other potential treatments, using a master protocol (SOLIDARITY) developed by WHO. This study (NCT04315948) is a multi-centre, adaptive, randomized, open clinical trial of the safety and efficacy of treatments of COVID-19 in hospitalized adults. Adults hospitalized for severe COVID-19 will be randomized to one of 4 treatment arms, including standard of care, remdesivir, lopinavir/r plus interferon β -1a and hydroxychloroquine.

In the meantime, EMA's human medicines committee (CHMP) has started a 'rolling review' of data. This speeds up the assessment of a promising investigational medicine during a public health emergency but does not imply that its benefits outweigh its risks. The EUA allows for the distribution and emergency use of remdesivir only for the treatment of COVID-19; remdesivir remains an investigational drug and has not been approved by FDA. The fact sheet for health care providers is found here: [FDA 2020](#).

Favipiravir

Favipiravir is another broad antiviral RdRp inhibitor that has been approved for influenza in Japan (but was never brought to the market) and other countries (Shiraki 2020). Favipiravir is converted into an active form intracellularly and recognized as a substrate by the viral RNA polymerase, acting like a chain terminator and thus inhibiting RNA polymerase activity (Delang 2018). In an *in vitro* study, this compound showed no strong activity against a clinical isolate of SARS-CoV-2 (Wang 2020). On February 14, however, a press release with promising results was published in Shenzhen (PR). In the absence of scientific data, favipiravir has been granted five-year approval in China under the trade name Favilavir® (in Europe: Avigan®). A loading dose of 2400 mg BID is recommended, following a maintenance dose of 1200–1800 mg QD. Potential drug-drug interactions (DDIs) have to be considered. As the parent drug undergoes metabolism in the liver mainly by aldehyde oxidase (AO), potent AO inhibitors such as cimetidine, amlodipine, or amitriptyline are expected to cause relevant DDIs (review: Du 2020), including foetal abnormalities in pregnant women

Clinical data: Uncontrolled data (Cai 2020) and preliminary results (press release) on encouraging results in 340 COVID-19 patients were reported from Wuhan and Shenzhen. With favipiravir, patients showed shorter periods of fever (2.5 versus 4.2 days), faster viral clearance (4 versus 11 days) and improvement in radiological findings (Bryner 2020). A first open-label randomized trial (RCT) was posted on March 26 (Chen 2020). This RCT was conducted in 3 hospitals from China, comparing arbidol and favipiravir in 236 patients with COVID-19 pneumonia. Primary outcome was the 7-day clinical recovery rate (recovery of fever, respiratory rate, oxygen saturation and cough relief). In “ordinary” COVID-19 patients (not critical), recovery rates were 56% with arbidol (n=111) and 71% (n=98) with favipiravir (p=0.02),

which was well tolerated, except for some elevated serum uric acid levels. However, it remains unclear whether these striking results are credible. In the whole study population, no difference was evident. Many cases were not confirmed by PCR. There were also imbalances between subgroups of “ordinary” patients. On May 26, the Japanese government postponed approving, after an interim analysis covering 40 patients by a third-party organization stated that it was “too soon to evaluate effectiveness”.

Other RdRp inhibitors

Some other compounds inhibiting RdRp have been discussed. Ribavirin is a guanosine analogue and RNA synthesis inhibitor that was used for many years for hepatitis C infection and is also thought to inhibit RdRp (Elfiky 2020). In SARS and MERS, ribavirin was mostly combined with lopinavir/ritonavir or interferon; however, a clinical effect has never been shown (Arabi 2017). Ribavirin is now available generically. Its use is limited by considerable side effects, especially anemia. Sofosbuvir is a polymerase inhibitor which is also used as a direct-acting agent in hepatitis C. It is usually very well tolerated. Modelling studies have shown that sofosbuvir could also inhibit RdRp by competing with physiological nucleotides for RdRp active site (Elfiky 2020). Sofosbuvir could be combined with HCV PIs. Among these, the fixed antiviral combinations with ledipasvir or velpatasvir could be particularly attractive as they may inhibit the both RdRp and protease of SARS-CoV-2 (Chen 2020). Studies are planned but not yet officially registered (assessed May 31).

Protease inhibitors

Lopinavir

This HIV protease inhibitor (PI) is thought to inhibit the 3-chymotrypsin-like protease of coronaviruses. Lopinavir/r is administered orally. To achieve appropriate plasma levels, it has to

be boosted with another HIV PI called ritonavir (usually indicated by “/r”: lopinavir/r). At least two case-control studies on SARS (Chan 2003, Chu 2004) and one prophylactic study on MERS (Park 2019) have indicated a beneficial effect, but the evidence remains poor. A small substudy indicated that SARS-CoV viral load seems to decrease more quickly with lopinavir than without (Chu 2004). However, all studies were small and non-randomized. It therefore remained unclear, whether all prognostic factors were matched appropriately. As with all HIV PIs, one should be always aware of drug-drug interactions. Ritonavir is a strong pharmacoenhancer. For example, tacrolimus has to be reduced by 10-100 fold to maintain concentration within the therapeutical range. In a case report, a woman with kidney transplantation was treated with lopinavir/r for COVID-19 while receiving full dose tacrolimus. Levels went incredibly high and were still above the therapeutical range 9 days after stopping both lopinavir/r and tacrolimus (Bartirolo 2020).

From the beginning of the pandemic, lopinavir/r has been widely used in clinical practice, despite the lack of any evidence (Chen 2020). For example, of all patients in the remdesivir trial NCT04257656, 18% were on lopinavir/r at baseline (Wang 2020).

Clinical data: In an early retrospective study on 280 cases, early initiation of lopinavir/r and/or ribavirin showed some benefits (Wu 2020). However, in a small study from Singapore study, lopinavir/r did not affect SARS-CoV-2 clearance in nasal swabs (Young 2020). There are two randomized clinical trials (RCT) published to date:

- The first open-label RCT in 199 adults hospitalized with severe COVID-19 did not find any clinical benefit with lopinavir/r treatment beyond standard care in patients receiving the drug 10 to 17 days after onset of illness (Cao 2020). The percentages of patients with detectable viral RNA at var-

ious time points were similar, suggesting no discernible effect on viral shedding.

- A Phase 2, multicentre, open-label RCT from Hong Kong randomized 127 patients with mild-to-moderate COVID-19 (median 5 days from symptom onset) to receive lopinavir/r only or a triple combination consisting lopinavir/r, ribavirin and interferon (Hung 2020). The results indicate that the triple combination can be beneficial when started early (see below, interferon). As there was no lopinavir/r-free control group, this trial does not prove lopinavir/r efficacy.

At least two studies suggested that lopinavir pharmacokinetics in COVID-19 patients may differ from those seen in HIV-infected patients. In both studies, very high concentrations were observed, exceeding those in HIV-infected patients by 2-3 fold (Schoergenhofer 2020, Gregoire 2020). However, concentrations of protein-unbound lopinavir achieved by current HIV dosing is probably still too low for inhibiting SARS-CoV-2 replication. The EC50 for HIV is much lower than for SARS-CoV-2. It remains to be seen whether these levels will be sufficient for (earlier) treatment of mild cases or as post-exposure prophylaxis. More than 30 clinical trials are ongoing. Lopinavir/r will be tested in WHO's huge SOLIDARITY trial.

Other PIs

For another HIV PI, the manufacturer Janssen-Cilag published a letter to the European Medical Agency on March 13, pointing out that “based on preliminary, unpublished results from a previously reported *in vitro* experiment, it is not likely darunavir will have significant activity against SARS-CoV-2 when administered at the approved safe and efficacious dose for the treatment of HIV-1 infection.” There is no evidence from both cell experiments or clinical observations that the drug has any prophylactic effect (De Meyer 2020, Härter 2020).

It is hoped that the recently published pharmacokinetic characterization of crystal structure of the main protease SARS-CoV-2 may lead to the design of optimized protease inhibitors (Zhang 2020). Virtual drug screening to identify new drug leads that target protease which plays a pivotal role in mediating viral replication and transcription, have already identified several compounds. Six compounds inhibited M(pro) with IC₅₀ values ranging from 0.67 to 21.4 μ M, among them with disulfiram and carmofur (a pyrimidine analogue used as an antineoplastic agent) two approved drugs (Jin 2020).

Antiviral entry inhibitors

Most coronaviruses attach to cellular receptors by their spike (S) protein. Within a few weeks, several groups have elucidated the entry of SARS-CoV-2 into the target cell (Hoffmann 2020, Zhou 2020). Similar to SARS-CoV, SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as a key receptor, a surface protein that is found in various organs and on lung AT2 alveolar epithelial cells. The affinity for this ACE-2 receptor appears to be higher with SARS-CoV-2 than with other coronaviruses. The hypothesis that ACE inhibitors promote severe COVID-19 courses through increased expression of the ACE2 receptor remains unproven (see clinical chapter).

Camostat

In addition to binding to the ACE2 receptor, priming or cleavage of the spike protein is also necessary for viral entry, enabling the fusion of viral and cellular membranes. SARS-CoV-2 uses the cellular protease transmembrane protease serine 2 (TMPRSS2). Compounds inhibiting this protease may therefore inhibit viral entry (Kawase 2012). The TMPRSS2 inhibitor camostat, which was approved in Japan for the treatment of chronic pancreatitis

(trade name: Foipán®), may block the cellular entry of the SARS-CoV-2 virus (Hoffmann 2020).

Clinical data: pending. At least five trials are ongoing. A Phase III study in the UK (named SPIKE1) in patients who exhibit symptoms but do not require hospitalization was announced at the end of May. Another Phase II study is underway in Denmark. A German study (CLOCC trial) which has been planned to start in June, comparing camostat and hydroxychloroquine, will have to deal with the disappointing results of HCQ (see below).

Umifenovir

Umifenovir (Arbidol®) is a broad-spectrum antiviral drug which is approved as a membrane fusion inhibitor in Russia and China for the prophylaxis and treatment of influenza. Chinese guidelines recommend it for COVID-19, according to a Chinese press release it is able to inhibit the replication of SARS-CoV-2 in low concentrations of 10-30 μ M (PR 2020).

Clinical data: In a small retrospective and uncontrolled study in mild to moderate COVID-19 cases, 16 patients who were treated with oral umifenovir 200 mg TID and lopinavir/r were compared with 17 patients who had received lopinavir/r as monotherapy for 5-21 days (Deng 2020). At day 7 (day 14), in the combination group, SARS-CoV-2 nasopharyngeal specimens became negative in 75% (94%), compared to 35% (53%) with lopinavir/r monotherapy. Chest CT scans were improving for 69% versus 29%, respectively. Similar results were seen in another retrospective analysis (Zhu 2020). However, a clear explanation for this remarkable benefit was not provided. Another retrospective study on 45 patients from a non-intensive care unit in Jinyintan, China failed to show any clinical benefit (Lian 2020). There is a preliminary report of a randomized study indicating a weaker effect of umifenovir compared to favipiravir (Chen 2020).

Hydroxychloroquine (HCQ) and Chloroquine (CQ)

Chloroquine is used for prevention and treatment of malaria and is effective (but not approved) as an anti-inflammatory agent for rheumatoid arthritis and lupus erythematosus. Hydroxychloroquine is approved for malaria and certain autoimmune diseases and is also better tolerated. Some lab experiments had suggested that HCQ and CQ might have some antiviral effects against SARS-CoV-2, due to an increase in the endosomal pH value, which disrupts the virus-cell fusion and some post-entry steps (Wang 2020, Yao 2020). An early enthusiastic mini-review stated that “results from more than 100 patients” showed that chloroquine phosphate would be able to alleviate the course of the disease (Gao 2020). Other experts, however, raised doubts (Touret 2020). A benefit of chloroquine would be the first positive signal, after decades of unsuccessfully studies conducted in a huge number of acute viral diseases. On March 17, a preliminary report from Marseille, France appeared to show some benefit in a small non-randomized study on 36 patients (Gautret 2020). Although this work lacked essential standards of data generation and interpretation (Kim 2020), someone’s swanky tweet on March 21 claiming that the combination of HCQ and azithromycin has “a real chance to be one of the biggest game changers in the history of medicine”, attracted world-wide attention and led to ten thousands of uncontrolled treatments. Moreover, many patients turned away from clinical trials of other therapies that would require them to give up chloroquine treatments. This has already prompted serious delays in trial enrolment, muddled efforts to interpret data and endangered clinical research (Ledford 2020). Some countries stockpiled CQ and HCQ, resulting in a shortage of these medications for those that need them for approved clinical indications. Only a few weeks later, we are now facing an overwhelming amount of data strongly arguing against any use of both HCQ and CQ.

Clinical data: There are no large RCT, comparing HCQ or CQ with placebo as treatment. However, growing data indicates that there is only low efficacy. If there is any. Some key studies arguing against HCQ during recent weeks

- In an observational study from New York City ([Geleris 2020](#)) of 1,376 consecutive hospitalized patients, 811 received HCQ (60% received also azithromycin). After adjusting for several confounders (HCQ patients were more severely ill at baseline), there was no significant association between HCQ use and intubation or death.
- Another retrospective cohort of 1,438 patients from 25 hospitals in the New York metropolitan region looked at 1,438 patients ([Rosenberg 2020](#)). In adjusted Cox models, compared with patients receiving neither drug, there were no significant differences in mortality for patients receiving HCQ + azithromycin, HCQ alone, or azithromycin alone. Cardiac arrest was significantly more likely seen with HCQ + azithromycin (adjusted OR 2.13).
- A randomized, Phase IIb clinical trial in Brazil allocated severe COVID-19 patients to receive high-dosage CQ (600 mg BID for 10 days) or low-dosage CQ (450 mg BID on day 1, QD for 4 days). The DSMB terminated the trial after 81/440 individuals had been enrolled ([Borba 2020](#)). By day 13 of enrolment, 6/40 patients (15%) in the low-dose group had died, compared with 16/41 (39%) in the high-dose group. Viral RNA was detected in 78% and 76%, respectively.
- In a retrospective study of 251 patients receiving HCQ plus azithromycin, extreme new QTc prolongation to > 500 ms, a known marker of high risk for torsade de pointes, had developed in 23% ([Chorin 2020](#)).
- In 150 patients with mainly persistent mild to moderate COVID-19, the probability of negative PCR conversion by 28 was 85.4% with HCQ, similar to that in the standard of care

group (81.3%) (Tang W 2020). Adverse events were recorded more frequently with HCQ (30% vs 9%, mainly diarrhea).

- Free plasma HCQ concentration achieved with HCQ doses tolerable for humans are probably too low to have any antiviral effects (Fan 2020).
- HCQ does not work as a prophylaxis. In total, 821 asymptomatic participants were randomized to receive hydroxychloroquine or placebo within 4 days after exposure (88% with a high-risk exposure). Incidence of confirmed SARS-CoV-2 was 11.8% with CQ and 14.3% with placebo. Side effects were more common with hydroxychloroquine than with placebo (40.1% vs. 16.8%), but no serious adverse reactions were reported (Boulware 2020).

The main conclusion of a recent review was that “there is insufficient and often conflicting evidence on the benefits and harms of using hydroxychloroquine or chloroquine to treat COVID-19. As such, it is impossible to determine the balance of benefits to harms”. There are no assessments of hydroxychloroquine or chloroquine for prophylaxis against COVID-19 (Hernandez 2020). No. 45 may continue to take it, but for other patients, there is no rationale outside of clinical trials.

Others

Baricitinib (Olumiant®) is a Janus-associated kinase (JAK) inhibitor approved for rheumatoid arthritis. Using virtual screening algorithms, baricitinib was identified as a substance that could inhibit ACE2-mediated endocytosis (Stebbing 2020). Like other JAK inhibitors such as fedratinib or ruxolitinib, signaling inhibition may also reduce the effects of the increased cytokine levels that are frequently seen in patients with COVID-19. There is some evidence that baricitinib could be the optimal agent in this group (Richardson 2020). Other experts have argued that the drug would be not an ideal option due the fact that baricitinib

causes lymphocytopenia, neutropenia and viral reactivation (Praveen 2020). However, several studies are underway in Italy and the US, among them a huge trial (ACTT-II), comparing bari-citinib and remdesivir to remdesivir alone in more than 1,000 patients.

Oseltamivir (Tamiflu®) is a neuraminidase inhibitor that is also approved for the treatment and prophylaxis of influenza in many countries. Like lopinavir, oseltamivir has been widely used for the current outbreak in China (Guan 2020). Initiation may be crucial immediately after the onset of symptoms. Oseltamivir is best indicated for accompanying influenza coinfection, which has been seen as quite common in MERS patients at around 30% (Bleibtreu 2018). There is no valid data for COVID-19. It is more than questionable whether there is a direct effect in influenza-negative patients with COVID-19 pneumonia. SARS-CoV-2 does not require neuramidases to enter target cells.

Immunomodulators

While antiviral drugs are most likely to prevent mild COVID-19 cases from becoming severe, adjuvant strategies will be particularly necessary in severe cases. Coronavirus infections may induce excessive and aberrant, ultimately ineffective host immune responses that are associated with severe lung damage (Channappanavar 2017). Similar to SARS and MERS, some patients with COVID-19 develop acute respiratory distress syndrome (ARDS), often associated with a cytokine storm (Mehta 2020). This is characterized by increased plasma concentrations of various interleukins, chemokines and inflammatory proteins.

Various host-specific therapies aim to limit the immense damage caused by the dysregulation of pro-inflammatory cytokine and chemokine reactions (Zumla 2020). Immunosuppressants, interleukin-1 blocking agents such as anakinra or JAK-2 inhibitors are also an option (Mehta 2020). These therapies may potentially act

synergistically when combined with antivirals. Numerous drugs are discussed, including those for lowering cholesterol, for diabetes, arthritis, epilepsy and cancer, but also antibiotics. They are said to modulate autophagy, promote other immune effector mechanisms and the production of antimicrobial peptides. Other immunomodulatory and other approaches in clinical testing include bevacizumab, brilacidin, cyclosporin, fedratinib (Wu 2020), fingolimod, lenadilomide and thalidomide, sildenafil, teicoplanin (Baron 2020), monoclonal antibodies (Shanmugaraj 2020) and many more. However, convincing clinical data is pending for most strategies.

Interferon

The interferon (IFN) response constitutes the major first line of defense against viruses. This complex host defense strategy can, with accurate understanding of its biology, be translated into safe and effective antiviral therapies. In a recent comprehensive review, the recent progress in our understanding of both type I and type III IFN-mediated innate antiviral responses against human coronaviruses is described (Park 2020).

In patients with coronaviruses such as MERS, however, interferon studies were disappointing. Despite impressive antiviral effects in cell cultures (Falzarano 2013), no convincing benefit was shown in clinical studies in combination with ribavirin (Omrani 2014, Shalhoub 2015, Arabi 2017).

Nevertheless, inhalation of interferon is still recommended as an option in Chinese COVID-19 treatment guidelines.

Clinical data: A Phase 2, multicentre, open-label RCT from Hong Kong randomized 127 patients with mild-to-moderate COVID-19 (median 5 days from symptom onset) to receive lopinavir/r only or a triple combination consisting lopinavir/r, ribavirin and interferon (Hung 2020). This trial indicates that the triple combination can be beneficial when started early. Combination thera-

py was given only in patients with less than 7 days from symptom onset and consisted of lopinavir/r, ribavirin (400 mg BID), and interferon beta-1b (1-3 doses of 8 Mio IE per week). Combination therapy led to a significantly shorter median time to negative results in nasopharyngeal swab (7 versus 12 days, $p = 0.001$) and other specimens. Clinical improvement was significantly better, with a shorter time to complete alleviation of symptoms and a shorter hospital stay. Of note, all differences were driven by the 76 patients who started treatment less than 7 days after onset of symptoms. In these patients, it seems that interferon made the difference. Up to now, this is the only larger RCT showing a virological response of a specific drug regimen.

Corticosteroids

Corticosteroids are often used, especially in severe cases. In the largest uncontrolled cohort study to date of 1,099 patients with COVID-19, a total of 19% were treated with corticosteroids, in severe cases almost half of all patients (Guan 2020). However, according to current WHO guidelines, steroids are not recommended outside clinical trials.

A systematic review of several observational SARS studies (Stockman 2006) yielded no benefit and various side effects (avascular necrosis, psychosis, diabetes). However, the use of corticosteroids COVID-19 is still very controversial (R Russell 2020, Shang 2020). In a retrospective study of 401 patients with SARS, it was found that low doses reduce mortality and are able to shorten the length of hospital stay for critically ill patients, without causing secondary infection and/or other complications (Chen 2006).

In another retrospective study involving a total of 201 COVID-19 patients, methylprednisolone reduced mortality in patients with ARDS (Wu 2020). One group, after reviewing 213 patients, postulated that an early short course of methylprednisolone in pa-

tients with moderate to severe COVID-19 may reduce escalation of care and improved clinical outcomes (Fadel 2020).

On the other hand, there is strong evidence of a delayed viral clearance (Ling 2020), which has also been observed with SARS (Stockman 2006). In a consensus statement by the Chinese Thoracic Society on February 8, corticosteroids should only be used with caution, after careful consideration, at low doses ($\leq 0.5\text{--}1$ mg/kg methylprednisolone or equivalent per day) and, last but not least, as short as possible (≤ 7 Days) (Zhao 2020).

Famotidine

Famotidine is a histamine-2 receptor antagonist that suppresses gastric acid production. It has an excellent safety profile. Initially it was thought to inhibit the 3-chymotrypsin-like protease (3CLpro), but it seems to act rather as an immune modulator, via its antagonism or inverse-agonism of histamine signalling. A retrospective study looked at 1,620 patients, including 84 patients (5.1%) who received different doses of famotidine within 24 hours of hospital admission (Freedberg 2020). After adjusting for baseline patient characteristics, use of famotidine remained independently associated with risk for death or intubation (adjusted hazard ratio 0.42, 95% CI 0.21-0.85) and this remained unchanged after careful propensity score matching to further balance the co-variables. Of note, there was no protective effect associated with use of PPIs. The maximum plasma ferritin value during the hospitalization was lower with famotidine, indicating that the drug blocks viral replication and reduces cytokine storm. Randomized clinical trials are underway.

Cytokine Blockers

The hypothesis that quelling the cytokine storm with anti-inflammatory therapies directed at reducing interleukin-6 (IL-6), IL-1, or even tumour necrosis factor TNF alpha, might be benefi-

cial has led to several ongoing trials. It is suggestive that interleukin blocking strategies might improve the hyperinflammatory state seen in severe COVID-19. A recent review on this strategy, however, was less enthusiastic and urged caution ([Remy 2020](#)). Past attempts to block the cytokine storm associated with other microbial infections and with sepsis have not been successful and, in some cases, have worsened outcomes. Moreover, there is concern that suppressing the innate and adaptive immune system to address increased cytokine concentrations, could enable unfettered viral replication, suppress adaptive immunity, and delay recovery processes. There is growing recognition that potent immunosuppressive mechanisms are also prevalent in such patients. Following, we will briefly discuss the evidence on cytokine blockers.

Anakinra

Anakinra is an FDA-approved treatment for rheumatoid arthritis and neonatal onset multisystem inflammatory disease. It is a recombinant human IL-1 receptor antagonist that prevents the binding of IL-1 and blocks signal transduction. Anakinra is thought to abrogate the dysfunctional immune response in hyperinflammatory COVID-19 and is currently being investigated in clinical trials.

Clinical data: Some case series have reported on encouraging results.

- A study from Paris, comparing 52 “consecutive” patients treated with anakinra with 44 historical patients. Admission to the ICU for invasive mechanical ventilation or death occurred in 25% of patients in the anakinra group and 73% of patients in the historical group. The treatment effect of anakinra remained significant in the multivariate analysis. Controlled trials are needed.

- A retrospective cohort study at the San Raffaele Hospital in Milan, Italy, including 29 patients with moderate-to-severe ARDS and hyperinflammation (serum C-reactive protein, CRP ≥ 100 mg/L) who were managed with non-invasive ventilation and HCQ and lopinavir/r (Cavalli 2020). At 21 days, treatment with high-dose anakinra was associated with reductions in CRP and progressive improvements in respiratory function in 21/29 (72%) patients.
- Another small case series of critically ill patients with secondary hemophagocytic lymphohistocytosis (sHLH) characterized by pancytopenia, hyper-coagulation, acute kidney injury and hepatobiliary dysfunction. At the end of treatment, ICU patients had less need for vasopressors and significantly improved respiratory function. Although 3/8 patients died, the mortality was lower than historical series of patients with sHLH in sepsis (Dimopoulos 2020).
- Clinical improvement in three patients with acute leukaemia and confirmed or suspected COVID-19 pneumonia with a life-threatening hyperinflammatory syndrome (Day 2020).

Tocilizumab

Tocilizumab (TCZ) is a monoclonal antibody that targets the interleukin-6 receptor. Tocilizumab (RoActemra® or Actemra®) is used for rheumatic arthritis and has a good safety profile. There is no doubt that TCZ should be reserved for patients with severe disease who have failed other therapies. However, some case reports have suggested that IL-6-blocking treatment given for chronic autoimmune diseases may even prevent the development of severe COVID-19 (Mihai 2020). The initial dose should be 4-8 mg/kg, with the recommended dosage being 400 mg (infusion over more than 1 hour). Controlled trials are underway (as of May 31, 46 trials at clinicaltrials.gov were listed, among them

14 Phase III studies) as well as for sarilumab (Kevzara[®]), another IL-6 receptor antagonist.

Clinical data: Some uncontrolled case series exist, many showing rapid relief of respiratory symptoms in some patients, as well as a resolution of fever and reduction in CRP following TCZ administration.

- 62 consecutive patients admitted to the Montichiari Hospital (Italy) with COVID-19 related pneumonia and respiratory failure (but not needing mechanical ventilation) received tocilizumab when the drug became available on March 12 (Capra 2020). Patients were compared with 23 “control” patients admitted before March 13th who were prescribed the standard therapy (HCQ, lopinavir/r). Patients receiving TCZ showed significantly greater survival rate, even after adjusting for baseline clinical characteristics. Only two out of 62 patients of the TCZ group and 11 out of 23 in the control group died. The respiratory function resulted improved in 64.8% of the observations in tocilizumab patients who were still hospitalized, whereas 100% of controls worsened and needed mechanical ventilation.
- Among 58 patients who received TCZ at a center in Barcelona, 8 (14%) died. Almost all (98%) received intravenous pulse therapy with steroids. There was a trend towards lower mortality when steroids were given before TCZ (Campins 2020).
- In a risk-adjusted Cox regression analysis of 31 hyperglycemic and 47 normoglycemic patients with severe COVID-19, TCZ in hyperglycemic patients failed to attenuate the risk of severe outcomes as it did in normoglycemic patients (Marfella 2020).
- Off-label use in 45 patients (most requiring high-flow oxygen supplementation or invasive ventilation) from Milan (Morena 2020). 14 died (27%). From baseline to day 7 after TCZ, however, a dramatic drop of body temperature and CRP value

with a significant increase in lymphocyte count was seen (Morena 2020).

Siltuximab

Siltuximab (Sylvant[®]) is another anti-IL-6-blocking agent. However, this chimeric monoclonal antibody targets interleukin-6 directly and not the receptor. Siltuximab has been approved for idiopathic multicentric Castleman's disease (iMCD). In these patients it is well tolerated.

Clinical data: First results of a pilot trial in Italy ("SISCO trial") have shown encouraging results. According to interim interim data, presented on April 2 from the first 21 patients treated with siltuximab and followed for up to seven days, one-third (33%) of patients experienced a clinical improvement with a reduced need for oxygen support and 43% of patients saw their condition stabilise, indicated by no clinically relevant changes (McKee 2020).

Passive immunization

A meta-analysis of observational studies on passive immunotherapy for SARS and severe influenza indicates a decrease in mortality, but the studies were commonly of low or very low quality and lacked control groups (Mair-Jenkins 2015). In MERS, fresh frozen convalescent plasma or immunoglobulin from recovered patients have been discussed (Zumla 2015, Arabi 2017). Recovered SARS patients develop a neutralizing antibody response against the viral spike protein (Liu 2006). Preliminary data indicate that this response also extends to SARS-CoV-2 (Hoffmann 2020), but the effect on SARS-CoV-2 was somewhat weaker. Others have argued that human convalescent serum could be an option for prevention and treatment of COVID-19 disease to be rapidly available when there are sufficient numbers of people who have recovered and can donate immunoglobulin-

containing serum (Casadevall 2020). Recently, an overview on current evidence of benefit, regulatory considerations, logistical work flow (recruitment of donors etc) and proposed clinical trials has been published (Bloch 2020). Passive immune therapy appears to be relatively safe. However, an unintended consequence of receiving convalescent plasma or globulins may be that recipients won't develop their own immunity, putting them at risk for reinfection. Other issues that have to be addressed in clinical practice (Kupferschmidt 2020) are plasma supply (may become a challenge), consistency (concentration differs) and rare but relevant risks (transfusion-related acute lung injury, in which transferred antibodies damage pulmonary blood vessels, or transfusion-associated circulatory overload).

Clinical data: Up to now, no larger controlled clinical trials in COVID-19 have been published. There are small case series:

- In 5 critically ill patients with COVID-19 and ARDS, administration of convalescent plasma was followed by improvement in their clinical status (Shen 2020). All 5 patients were receiving mechanical ventilation at the time of treatment and all had received antiviral agents and methylprednisolone.
- In another pilot study, a single dose (200 mL) of convalescent plasma was given to 10 patients (9 treated with umifenovir, 6 with methylprednisolone, 1 with remdesivir). In all 7 patients with viremia, serum SARS-CoV-2 RNA decreased to an undetectable level within 2-6 days (Duan 2020). Meanwhile, clinical symptoms and paraclinical criteria rapidly improved - within three days.
- In 25 patients with severe and/or life-threatening COVID-19 disease enrolled at Houston, convalescent plasma was safe. By day 14 post-transfusion, 19 (76%) patients had at least a 1-point improvement in clinical status and 11 were discharged (Salazar 2020).

- Don't be too late: Of 6 patients with respiratory failure receiving convalescent plasma at a median of 21 days after first detection of viral shedding, all tested RNA negative by 3 days after infusion. However, 5 eventually died ([Zeng 2020](#)).

On March 26, the FDA has approved the use of plasma from recovered patients to treat people who are critically ill with COVID-19 ([Tanne 2020](#)). It's now time for larger and controlled studies.

Monoclonal antibodies

As long as all other therapies fail or have only modest effects, monoclonal neutralizing antibodies are the hope for the near future. There is no doubt that antibodies with high and broad neutralizing capacity, many of them directed to the receptor binding domain (RBD) of SARS-CoV-2, are promising candidates for prophylactic and therapeutic treatment. On the other hand, these antibodies also have to go through all phases of clinical trial testing programs, which will take time. Safety and tolerability in particular is an important issue. The production of larger quantities is also likely to cause problems. No antibody has been tested in humans to date. However, some are very promising. Key papers:

- The first report of a human monoclonal antibody that neutralizes SARS-CoV-2 ([Wang 2020](#)). 47D11 binds a conserved epitope on the spike RBD explaining its ability to cross-neutralize SARS-CoV and SARS-CoV-2, using a mechanism that is independent of receptor-binding inhibition. This antibody could be useful for development of antigen detection tests and serological assays targeting SARS-CoV-2.
- Fantastic study identifying 14 potent neutralizing antibodies by high-throughput single B cell RNA-sequencing from 60 convalescent patients ([Cao 2020](#)). The most potent one, BD-368-2, exhibited an IC₅₀ of 15 ng/mL against SARS-CoV-2.

This antibody displayed strong therapeutic and prophylactic efficacy in mice, the epitope overlaps with the ACE2 binding site. Time to go into the clinic!

- Isolation and characterization of 206 RBD-specific monoclonal antibodies derived from single B cells of eight SARS-CoV-2 infected individuals. Some antibodies showed potent anti-SARS-CoV-2 neutralization activity that correlates with their competitive capacity with ACE2 for RBD binding (Ju 2020).
- Four human-origin monoclonal antibodies were isolated from a convalescent patient, all of which display neutralization abilities. B38 and H4 blocked the binding between virus S-protein RBD and cellular receptor ACE2. A competition assay indicates their different epitopes on the RBD. In a mouse model, both antibodies reduced virus titers in infected lungs. The RBD-B38 complex structure revealed that most residues on the epitope overlap with the RBD-ACE2 binding interface, explaining the blocking effect and neutralizing capacity (Wu 2020).

Outlook

It is hoped that at least some of the options given in this overview will show positive results over time. It is also important that in this difficult situation, despite the immense pressure, the basic principles of drug development and research including repurposing are not abandoned.

Four different options, namely lopinavir/r, alone and in combination with interferon, remdesivir and (hydroxy) chloroquine will be tested in the SOLIDARITY study launched on March 18 by the WHO. Results of this large-scale, pragmatic trial will generate the robust data we need, to show which treatments are the most effective (Sayburn 2020).

So in the present dark times, which are the best options to offer patients? There is currently no evidence from controlled clinical trials to recommend a specific treatment for SARS-CoV-2 coronavirus infection. Guidelines do not help, especially those concluding that evidence is insufficient and that “all patients should be treated in controlled randomized trials”. Moreover, on the day of their publication, many guidelines are outdated. However, after reviewing all these studies until May 31, we would recommend reviewing the following treatment options, considering the severity of the disease:

Hospital, severe COVID-19

- In the clinic, use remdesivir if available and as soon as possible
- In patients with severe COVID-19, consider tocilizumab, anakinra and corticosteroids (short)

Outpatient, mild to moderate COVID-18

- Daily infusions of remdesivir are not feasible (and will not be approved)
- HCQ and CQ should no longer be used (too many side effects)
- Lopinavir is still an (useless) option, but interactions and gastrointestinal side effects have to be considered
- Famotidin: why not? Potential harm seems to be limited
- Interferon may work, if given early (optimal usage is unclear)

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9. Severe COVID

This chapter about severe COVID-19 in the hospital/ICU will be published soon. In the meantime, please find the following recommendations and key papers.

Checklists for hospitals

European Centre for Disease Prevention and Control. **Checklist for hospitals preparing for the reception and care of coronavirus 2019 (COVID-19) patients.** ECDC: Stockholm; 2020.
<https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-checklist-hospitals-preparing-reception-care-coronavirus-patients.pdf>

Patient admission to ICUs

Swiss Society Of Intensive Care Medicine. **Recommendations for the admission of patients with COVID-19 to intensive care and intermediate care units (ICUs and IMCUs).** Swiss Med Wkly. 2020 Mar 24. Fulltext:
<https://doi.org/10.4414/smw.2020.20227>

Management of critically ill patients

Thomas-Ruddel D, Winning J, Dickmann P, et al. **Coronavirus disease 2019 (COVID-19): update for anesthesiologists and intensivists March 2020.** Anaesthesist. 2020 Mar 24. Fulltext: <https://doi.org/10.1007/s00101-020-00760-3>

Outstanding update for anesthesiologists and those working in intensive care.

Sorbello M, El-Boghdady K, Di Giacinto I, et al. **The Italian COVID-19 outbreak: experiences and recommendations from clinical practice.** Anaesthesia. 2020 Mar 27. PubMed: <https://pubmed.gov/32221973>. Fulltext: <https://doi.org/10.1111/anae.15049>

Detailed practical recommendations, based on experiences in Italy. Key elements of clinical management, airway management, personal protective equipment and non-technical aspects.

Poston JT, Patel BK, Davis AM. **Management of Critically Ill Adults With COVID-19.** JAMA. 2020 Mar 26. Fulltext: <https://doi.org/10.1001/jama.2020.4914>

Short recommendations, made by the Surviving Sepsis Campaign.

More reviews, overviews of specific issues of critically ill patients

Berlin DA, Gulick RM, Martinez FJ. **Severe Covid-19.** May 15, 2020. DOI: 10.1056/NEJMcp2009575. Full-text: https://www.nejm.org/doi/full/10.1056/NEJMcp2009575?query=featured_home

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Recommendations for conducting autopsies

10. Comorbidities

Hundreds of articles have been published over the last few weeks, making well-meaning attempts to determine whether patients with different comorbidities are more susceptible for SARS-CoV-2 infection or at higher risk for severe disease. This deluge of scientific publications has resulted in worldwide uncertainty. For a number of reasons, many studies must be interpreted with extreme caution.

First, in many articles, the number of patients with specific comorbidities is low. Small sample sizes preclude accurate comparison of COVID-19 risk between these patients and the general population. They may also overestimate mortality, especially if the observations were made in-hospital (reporting bias). Moreover, the clinical manifestation and the relevance of a condition may be heterogeneous. Is the hypertension treated or untreated? What is the stage of the COPD, only mild or very severe with low blood oxygen levels? Is the “cancer” cured, untreated or actively being treated? Are we talking about a seminoma cured by surgical orchiectomy years ago or about palliative care for pancreatic cancer? What is a “former” smoker: someone who decided to quit 20 years ago after a few months puffing during adolescence or someone with 40 package-years who stopped the day before his lung transplantation? Does “HIV” mean a well controlled infection while on long-lasting, successful antiretroviral therapy or an untreated case of AIDS? Unfortunately, many researchers tend to combine these cases, in order to get larger numbers and to get their paper published.

Second, there are numerous confounding factors to consider. In some case series, only symptomatic patients are described, in others only those who were hospitalized (and who have *per se* a higher risk for severe disease). In some countries, every patient with SARS-CoV-2 infection will be hospitalized, in others only

those with risk factors or with severe COVID-19. Testing policies vary widely between countries. The control group (with or without comorbidities) is not always well-defined. Samples may not be representative, risk factors not correctly taken into account. Sometimes, there is incomplete information about age distribution, ethnicity, comorbidities, smoking, drug use and gender (there is some evidence that, in female patients, comorbidities have no or less impact on the course of the disease, compared to male (Meng 2020)). All these issues present important limitations and only a few studies have addressed all of them.

Third, comorbidity papers have led to an information overload. Yes, virtually every medical discipline and every specialist has to cope with the current pandemic. And yes, everybody has to be alert these days, psychiatrists as well as esthetic surgeons. Hundreds of guidelines or position papers have been published in recent weeks, trying to thoughtfully balance fear of COVID-19 against the dire consequences of not treating other diseases than COVID-19 in an effective or timely manner – and all this in the absence of data. On May 15, a PubMed search yielded 530 guidelines or considerations about specific diseases in the context of COVID-19, among them those for grade IV glioma (Bernhardt 2020, bottom line: do not delay treatment), but also for dysphonia and voice rehabilitation (Mattei 2020: can be postponed), infantile hemangiomas (Frieden 2020: use telehealth), ocular allergy (Leonardi 2020: very controversial), high resolution anoscopy (Mistrangelo 2020: also controversial), migraine management (Szperka 2020: use telehealth) and breast reconstruction (Salgarello 2020: defer “whenever possible”), to name just a few.

These recommendations are usually not helpful. They apply for a few weeks, during acute health crisis scenarios as seen in overwhelmed health care systems in Wuhan, Bergamo, Madrid or New York. In other cities or even a few weeks later, proposed algorithms are already outdated. And nobody needs a 60-page

recommendation, concluding that “clinical judgment and decision making should be exercised on a case-by-case basis”. However, some important papers have been published during the last months, a couple of them with very helpful data, supporting the management of patients with comorbidities. In the following, we will briefly go through these.

Hypertension and cardiovascular comorbidities

From the beginning of the pandemic, hypertension and/or cardiovascular disease (CVD) have been identified as potential risk factors for severe disease and death (at least two studies had performed a multivariate analysis (Table 1)). However, all studies were retrospective, included only hospitalized patients and did not distinguish between uncontrolled and controlled hypertension or used different definitions for CVD. Multivariate analyses adjusting for confounders were performed in only a few studies. Moreover, different outcomes and patient groups were analyzed.

According to some experts, current data do not necessarily imply a causal relationship between hypertension and severity of COVID-19. It is also “unclear whether uncontrolled blood pressure is a risk factor for acquiring COVID-19, or whether controlled blood pressure among patients with hypertension is or is not less of a risk factor” (Schiffirin 2020). The same applies to CVD, with the difference that the numbers here are even lower.

From a mechanistic point of view, however, it seems very plausible that patients with underlying cardiovascular diseases and pre-existing damage to blood vessels such as atherosclerosis may face higher risks for severe diseases. During recent weeks, it has become clear that SARS-CoV-2 may directly or indirectly attack the heart, kidney and blood vessels.

Table 1. Hypertension in larger cohort studies, prevalence and outcome

Study	Setting	Hypertension present?	Multivariate, hazard or odds ratio (95% CI) for endpoint
Wang 2020	344 ICU pts, Tongji, China	Survivors vs Non-Survivors: 34 vs 52%	Not done
Grasselli 2020	521 ICU pts, 72 hospitals in Italy	Discharge from ICU vs death at ICU: 40 vs 63%	Not done
Guan 2020	1,099 hospitalized pts, 522 hospitals in China	Non-severe disease vs severe: 13 vs 24%	Not done
Zhou 2020	191 hospitalized pts from Jinyintan and Wuhan	Survivors vs Non-Survivors: 23 vs 48%	Not done
Shi 2020	487 hospitalized pts in Zhejiang Province	Non-severe disease at admission vs severe: 17 vs 53%	OR 2.7 (1.3-5.6) for severe disease at admission
Guan 2020	1,590 hospitalized pts, 575 hospitals in China	Non-severe vs severe courses: 13 vs 33%	HR 1.6 (1.1-2.3) for severe course (ICU, IMV, death)
Goyal 2020	393 hospitalized pts, 2 hospitals in New York	No IMV vs IMV during stay: 48 vs 54%	Not done

IMV invasive mechanical ventilation, ICU intensive care units

Various cardiac manifestations of COVID-19 do occur contemporarily in many patients (see chapter Clinical Manifestations). Infection may lead to cardiac muscle damage, blood vessel constriction and to elevated levels of inflammation-inducing cytokines. These direct and indirect adverse effects of the virus may be especially deleterious in those with already established heart disease. During the next months, we will learn a lot more about the role and contributions of arteriosclerosis in the pathogenesis of COVID-19.

Treatment of hypertension during the pandemic

There has hardly been a topic in the last months that has kept doctors and their patients as busy as the question of whether antihypertensive drugs such as ACE inhibitors (ACEIs) or angiotensin-receptor blockers (ARBs) can cause harm to patients. The uncontrolled observations of increased mortality risk in patients with hypertension, CVD (see above) and diabetes raised concerns. These conditions share underlying renin-angiotensin-aldosterone system pathophysiology that may be clinically insightful. In particular, activity of the angiotensin-converting enzyme 2 (ACE2) is dysregulated (increased) in cardiovascular disease ([Vaduganathan 2020](#)). As SARS-CoV-2 cell entry depends on ACE2 ([Hoffmann 2020](#)), increased ACE2 levels may increase the virulence of the virus within the lung and heart.

ACEIs or ARBs may alter ACE2, and variation in ACE2 expression may in part be responsible for disease virulence. However, the first substantial study to examine the association between plasma ACE2 concentrations and the use of ACEIs/ARBs does not support this hypothesis: in two large cohorts from the pre-COVID-19 era, plasma concentrations of ACE2 were markedly higher in men than in women, but not with ACEI/ARB use ([Sama 2020](#)). A recent review of 12 animal studies and 12 human studies overwhelmingly implies that administration of both drug classes does not increase ACE2 expression ([Sriram 2020](#)).

However, some concerns on deleterious effects remain and some media sources and even scientific papers have called for the discontinuation of these drugs. This is remarkable as clinical data actually points in the opposite direction. Some small retrospective studies from China have shown no negative effect ([Meng 2020](#), [Yang 2020](#)). In the largest study, 188 patients taking ACEI/ARBs were compared with 940 patients who did not use them. Of note, unadjusted mortality rate was lower in the

ACEI/ARB group (3.7% vs. 9.8%) and a lower risk was also found in a multivariate Cox model (Zhang 2020).

By early May, two large studies were published in the NEJM (a third was later retracted). Although both were observational (with the possibility of confounding), their message was consistent - none showed any evidence of harm (Jarcho 2020). One study analyzed 2,573 COVID-19 patients with hypertension from New York City, among them 25% with severe disease (Reynolds 2020). After looking at different classes of antihypertensive medications - ACE inhibitors, ARBs, beta-blockers, calcium-channel blockers, and thiazide diuretics, the authors ruled out any substantial difference in the likelihood of severe COVID-19, with at least 97.5% certainty for all medication classes.

The second study looked at a possible independent relationship between ACEI/ARBs and the susceptibility to COVID-19 (Mancia 2020). The authors matched 6,272 Italian cases (positive for SARS-CoV-2) with 30,759 beneficiaries of the Regional Health Service (controls) according to sex, age, and municipality of residence. There was no evidence that ACE inhibitors or ARBs modify susceptibility to COVID-19. The results applied to both sexes as well as to younger and older persons.

In conclusion, ACE inhibitors and/or ARBs should not be discontinued (Bavishi 2020, Sriram 2020, Vaduganathan 2020). At least four registered randomized trials plan to evaluate ACEIs and ARBs for treatment of COVID-19 (Mackey 2020). According to a brief review, adjuvant treatment and continuation of pre-existing statin therapy could improve the clinical course of patients with COVID-19, either by their immunomodulatory action or by preventing cardiovascular damage (Castiglion 2020).

Treatment of coronary heart disease during the pandemic

Myocardial injury, evidenced by elevated cardiac biomarkers, was recognized among early cases and myocardial infarction (STEMI or NSTEMI) and may represent the first clinical manifestation of COVID-19 (Reviews: [Bonow 2020](#), [Valente 2020](#)). Of note, a culprit lesion is often not identifiable by coronary angiography. In a study of 28 patients with STEMI, this was the case in 39% ([Stefanini 2020](#)). According to the authors, a dedicated diagnostic pathway should be delineated for COVID-19 patients with STEMI, aimed at minimizing procedural risks and healthcare providers' risk of infection. There are already preliminary reports on a significant decline of 32% in the number of percutaneous coronary interventions for acute coronary syndromes ([Piccolo 2020](#)). Other authors have suggested that, in settings with limited resources to protect the work force, fibrinolytic therapies may be preferred over primary percutaneous coronary interventions ([Daniels 2020](#)).

Of note, several studies have found a spectacular drop in admissions for STEMI during the peak of the epidemic. In France a steep decline of 25% was found for both acute (< 24hrs) and late presentation (> 24 hrs) STEMI ([Rangé 2020](#)). Similar observations have been made in Italy ([De Filippo 2020](#)) and the US ([Solomon 2020](#)). Possible explanations for this phenomenon may be patients' fear of coming to the hospital or disturbing busy caregivers, especially in the case of mild STEMI clinical presentation. Other hypothetical reasons are reduced air pollution, better adherence to treatment, limited physical activity or absence of occupational stress during lockdown. However, there is some evidence that the lower incidence does not reflect a true decline but just one more collateral damage of the pandemic. For example, Italian researchers have found a 58% increase of out-of-hospital

cardiac arrests in March 2020 compared to the same period in 2019 (Baldi 2020).

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Diabetes mellitus

Diabetes mellitus is a chronic inflammatory condition characterized by several macrovascular and microvascular abnormalities. As with hypertension and CVD, many of the above cited studies have also revealed that diabetic patients were overrepresented among the most severely ill patients with COVID-19 and those succumbing to the disease. Current data suggest that diabetes in patients with COVID-19 is associated with a two-fold increase in mortality as well as severity of COVID-19, as compared to non-diabetics. In a meta-analysis of 33 studies and 16,003 patients

(Kumar 2020), diabetes was found to be significantly associated with mortality of COVID-19 with a pooled odds ratio of 1.90 (95% CI: 1.37-2.64). Diabetes was also associated with severe COVID-19 and a pooled odds ratio of 2.75 (95% CI: 2.09-3.62). The pooled prevalence of diabetes in patients with COVID-19 was 9.8% (95% CI: 8.7%-10.9%). However, it is too early to whether diabetes is acting as an independent factor responsible for COVID severity and mortality or if it is just a confounding factor.

The hitherto by far largest retrospective study on the impact of typ 2 diabetes (T2D) has carefully analyzed 7,337 cases of COVID-19 in Hubei Province, China, among them 952 with pre-existing T2D (Zhu 2020). The authors found that subjects with T2D required more medical interventions and had a significantly higher mortality (7.8% versus 2.7%; adjusted hazard ratio, 1.49) and multiple organ injury than non-diabetic individuals. Of note, well-controlled blood glucose was associated with markedly lower mortality (in-hospital death rate 1.1% versus 11.0%) compared to individuals with poorly controlled blood glucose.

A recent review has made some suggestions on the possible pathophysiological mechanisms of the relationship between diabetes and COVID-19, and its management (Hussain 2020). Rigorous glucose monitoring and careful consideration of drug interactions might attenuate worsening of symptoms and adverse outcomes. Some treatment strategies for COVID-19 such as steroids and lopinavir/r bear a risk for hyperglycemia. On the other hand, hydroxychloroquine may improve glycemic control in decompensated, treatment-refractory patients with diabetes (Gerstein 2002, Rekedal 2010). However, it remains unclear which COVID-19 treatment strategy works best and if treatment of diabetic patients have to be different from those without diabetes. It is also unclear whether specific diabetes drugs such as DPP4 inhibitors increase or decrease the susceptibility or severity of SARS-CoV-2 infection.

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COPD and smoking

Chronic Obstructive Pulmonary Disease (COPD) is a common and preventable dysfunction of the lung associated with limitation in airflow. It is a complex disease associated with abnormalities of the airway and/or alveoli which is predominantly caused by exposure to noxious gases and particulates over a long period. A meta-analysis of 15 studies, including a total of 2,473 confirmed COVID-19 cases showed that COPD patients were at a higher risk of more severe disease (calculated RR 1.88) and with 60% higher mortality (Alqahtani 2020). Unfortunately, the numbers in this review were very small and only 58 (2.3%) had COPD.

A meta-analysis of 5 early studies comprising 1,399 patients observed only a trend but no significant association between active

smoking and severity of COVID-19 (Lippi 2020). However, other authors have emphasized that current data do not allow to draw firm conclusions about the association of severity of COVID-19 with smoking status (Berlin 2020). In a more recent review, current smokers were 1.45 times more likely to have severe complications compared to former and never smokers. Current smokers also had a higher mortality rate (Alqahtani 2020).

Ever-smoking significantly and substantially increased pulmonary ACE2 expression by 25% (Cai 2020). The significant smoking effect on ACE2 pulmonary expression may suggest an increased risk for viral binding and entry of SARS-CoV-2 in lungs of smokers. Cigarette smoke triggers an increase in ACE2 positive cells by driving secretory cell expansion (Smith 2020). The overabundance of ACE2 in the lungs of smokers may partially explain a higher vulnerability of smokers.

However, it's not that easy – both quitting smoking and finding clinical correlations to the above cell experiments. Within a surveillance centre primary care sentinel network, multivariate logistic regression models were used to identify risk factors for positive SARS-CoV-2 tests (Lusignan 2020). Of note, active smoking was associated with decreased odds (yes, decreased: adjusted OR 0.49, 95% CI 0.34–0.71). According to the authors, their findings should not be used to conclude that smoking prevents SARS-CoV-2 infection, or to encourage ongoing smoking. Several explanations are given, such as selection bias (smokers are more likely to have a cough, more frequent testing could increase the proportion of smokers with negative results). Active smoking might also affect RT-PCR test sensitivity.

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HIV infection

HIV infection is of particular interest in the current crisis. First, many patients take antiretroviral therapies that are thought to have some effect against SARS-CoV-2. Second, HIV serves as a model of cellular immune deficiency. Third, and by far the most important point, the collateral damage caused by COVID-19 in the HIV population may be much higher than that of COVID-19 itself.

Inexplicably, information on the HIV population is still scarce. However, preliminary data suggest no elevated incidence of COVID-19. In 5,700 patients from New York, only 43 (0.8%) were found to be HIV-positive (Richardson 2020). Similar findings were reported from Chicago (Ridgeway 2020). In Barcelona where a local protocol included HIV serology for all hospitalized COVID-19 patients, 32/2102 (1.5%) were HIV-infected, among

them only one single new HIV diagnosis (Miro 2020). Given the fact that HIV+ patients may be at higher risk for other infectious diseases such as STDs, these percentages were so low that some experts have already speculated on potential “protective” factors (i.e., antiviral therapies or immune activation). Moreover, a defective cellular immunity could paradoxically be protective for severe cytokine dysregulation, preventing the cytokine storm seen in severe COVID-19 cases.

Appropriately powered and designed studies that are needed to draw conclusions on the effect of COVID-19 are still lacking. However, our own retrospective analysis of 33 confirmed SARS-CoV-2 infections between March 11 and April 17 in 12 participating German HIV centers revealed no excess morbidity or mortality (Haerter 2020). The clinical case definition was mild in 25/33 cases (76%), severe in 2/33 cases (6%), and critical in 6/33 cases (18%). At the last follow up, 29/32 of patients with documented outcome (90%) had recovered. Three out of 32 patients had died. One patient was 82 years old, one had a CD4 T cell count of 69/ μ l and one suffered from several comorbidities. A similar observation was made in Milan, Italy, where 45/47 patients with HIV and COVID-19 (only 28 with confirmed SARS-CoV-2 infection) recovered (Gervasoni 2020). In another single center study from Madrid on 51 HIV patients with COVID-19 (35 confirmed cases), six patients were critically ill and two died (Vizcarra 2020).

In these studies, as in our cohort, severe immune deficiency was rare. The last median CD4 count was 670/ μ l (range, 69 to 1715) and in 30/32 cases in our cohort, the latest HIV RNA was below 50 copies/mL (Härter 2020). It remains to be seen whether HIV+ patients with uncontrolled viremia and/or low CD4 cells are at higher risk for severe disease. It is also unclear whether immunity after infection remains impaired. However, there are case reports on delayed antibody response in HIV+ patients (Zhao 2020).

Another issue making HIV+ patients an interesting population is a potential effect of antiretroviral therapies against SARS-CoV-2. For lopinavir/r, some reports on beneficial effects in patients with SARS, MERS and COVID-19 exist, but the evidence remains poor. Several studies on lopinavir are still underway (see [Treatment chapter](#)). According to both the US DHHS and EACS statement, an ART regimen should not be changed to include a PI to prevent or treat COVID-19 ([EACS 2020](#), [US 2020](#)). In our cohort, 4/33 (12%) patients were on darunavir when they developed COVID-19 symptoms. In the Milan Cohort, the rate of patients on a PI was 11% ([Gervasoni 2020](#)). Both studies indicate that PIs do not protect from SARS-CoV-2 infection. Beside the PI, we did not find any clear evidence for a protective effect of tenofovir. Tenofovir alafenamide has some chemical similarities to [remdesivir](#) and has been shown to bind to SARS-CoV-2 RNA polymerase (RdRp) with binding energies comparable to those of native nucleotides, similar to remdesivir. Consequently, tenofovir has recently been suggested as a potential treatment for COVID-19 ([Elfiky 2020](#)). In Spain, a large randomized Phase III placebo-controlled study (EPICOS, NCT04334928) compares the use of tenofovir disoproxil fumarate/emtricitabine, hydroxychloroquine or the combination of both versus placebo as prophylaxis for COVID-19 in healthcare workers. Our observation that the majority (22/33) of HIV+ patients with COVID-19 were treated with tenofovir, including those developing severe or critical disease, indicate no or only minimal clinical effect against SARS-CoV-2 ([Härter 2020](#)). In the cohorts from Milan and Madrid, there was no evidence that any specific antiretroviral drug (such as tenofovir or PIs) affected COVID-19 susceptibility or severity ([Gervasoni 2020](#), [Vizcarra 2020](#)).

The most serious concern regarding HIV, however, is the collateral damage induced by COVID-19. In Western countries, there exist few reports of HIV+ patients having problems in gaining access to their HIV medications or having trouble taking them

due to COVID-19 or the plans to manage it (Sanchez 2020). In contrast, disruption to delivery of health care in sub-Saharan African settings could well lead to adverse consequences beyond those from COVID-19 itself. Lockdown, transport restrictions and fear of coronavirus infection have already led to a dramatic drop in HIV and TB patients collecting medication in several African countries (Adepoju 2020). Using five different existing mathematical models of HIV epidemiology and intervention programmes in sub-Saharan Africa, investigations have already estimated the impact of different disruptions to HIV prevention and treatment services. Predicted average relative excess in HIV-related deaths and new HIV infections (caused by unsuppressed HIV RNA during treatment interruptions) per year over 2020-2024 in countries in sub-Saharan Africa that would result from 3 months of disruption of HIV-specific services, were 1.20-1.27 for death and 1.02-1.33 for new infections, respectively. A 6-month interruption of ART would result in over 500,000 excess HIV deaths in sub-Saharan Africa (range of estimates 471,000 - 673,000). Disrupted services could also reverse gains made in preventing mother-to-child transmission. According to WHO, there is a clear need for urgent efforts to ensure HIV service continuity and preventing treatment interruptions due to COVID-19 restrictions in sub-Saharan Africa.

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Immunosuppression (other than HIV)

Immunosuppression may bear a higher risk for SARS-CoV-2 infection and severe COVID-19. But the story is not that simple. Neither is it clear what immunosuppression actually means, nor are the available data sufficient to draw any conclusion. We just don't know enough. Nevertheless, some authors are trumpeting the news that there is an increased risk. A bad example? A systematic review and meta-analysis on 8 studies and 4,007 patients came to the conclusion that "immunosuppression and immunodeficiency were associated with increased risk of severe COVID-19 disease, although the statistical differences were not significant" (Gao 2020). The authors also state that "in response to the COVID-19 pandemic, special preventive and protective measures should be provided." There is null evidence for this impressive statement. The total number of patients with immunosuppression in the study was 39 (without HIV: 11!), with 6/8 studies describing less than 4 patients with different modalities of immunosuppression.

Despite the large absence of data, numerous viewpoints and guidelines have been published on how to manage immunosuppressed patients that may be more susceptible to acquire COVID-19 infection and develop severe courses. There are recommendations for intranasal corticosteroids in allergic rhinitis (Bousquet 2020), immunosuppressants for psoriasis and other cutaneous diseases (Conforti 2020, Torres 2020)), rheumatic diseases (Favalli 2020, Figueroa-Parra 2020) or inflammatory bowel diseases (Kennedy 2020, Pasha 2020). The bottom line of these heroic attempts to balance the risk of immune-modifying drugs with the risk associated with active disease: what is generally needed, has to be done (or to be continued). Exposure prophylaxis is important.

However, two studies have indeed found evidence for deleterious effects of glucocorticoids, indicating that these drugs should be given with particular caution these days. The largest study published to date, analysed 525 patients with inflammatory bowel disease (IBD) from 33 countries (Brenner 2020). Thirty-seven patients (7%) had severe COVID-19, and 16 patients died (3% case fatality rate). Risk factors for severe COVID-19 among IBD patients included increasing age, ≥ 2 comorbidities, systemic corticosteroids (adjusted odds ratio 6.9, 95% CI 2.3-20.5), and sulfasalazine or 5-aminosalicylate use (aOR 3.1, 95% CI 1.3-7.7). Notably, TNF antagonist treatment was not associated with severe COVID-19. Another larger case series looked at 86 patients with immune-mediated inflammatory disease and symptomatic COVID-19, among them 62 receiving biologics or Janus kinase (JAK) inhibitors (Haberman 2020). The percentage of patients who were receiving biologics or JAK inhibitors at baseline was higher among the ambulatory than among the hospitalized patients. In contrast, hospitalization rates were higher in patients treated with oral glucocorticoids, hydroxychloroquine and methotrexate.

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Transplantation

During a health crisis such as the COVID pandemic, it is crucial to carefully balance cost and benefits in performing a transplantation (Andrea 2020). There is no doubt that the current situation has deeply affected organ donation and that this represents an important collateral damage of the pandemic. All Eurotransplant countries have implemented preventive screenings policies for potential organ donors. For detailed information on the national policy, please visit <https://www.eurotransplant.org/2020/04/07/covid-19-and->

[organ-donation/](#). Preliminary data indicate a significant reduction in transplantation rates even in regions where COVID-19 cases are low, suggesting a global and nationwide effect beyond the local COVID-19 infection prevalence ([Loupy 2020](#)). During March and April, the overall reduction in deceased donor transplantations since the COVID-19 outbreak was 91% in France and 51% in the USA, respectively. In both France and the USA, this reduction was mostly driven by kidney transplantation, but a substantial effect was also seen for heart, lung, and liver transplants, all of which provide meaningful improvement in survival probability.

Solid organ transplant recipients are generally at higher risk for complications of respiratory viral infections (in particular influenza), due to their chronic immunosuppressive regimen, and this may hold particularly true for SARS-CoV-2 infection. The first larger cohort of COVID-19 in transplant recipients from the US indeed indicated that transplant recipients appear to have more severe outcomes ([Pereira 2020](#)). Of 90 patients (median age of 57 years), 46 were kidney recipients, 17 lung, 13 liver, 9 heart and 5 dual-organ transplants. Sixteen patients died (18% overall, 24% of hospitalized, 52% of ICU). It remains unclear whether these high mortality and morbidity rates derived from reporting or selection bias. However, a single center experience with 36 kidney transplant recipients found even higher rates. After 21 days, 10/36 had died ([Akalın 2020](#)). Patients appear to have less fever as an initial symptom, lower CD3/4/8 cell counts and more rapid clinical progression. In a case series of 28 patients who had received heart transplant in a large academic center in New York, 22 patients (79%) were hospitalized. At the end of the follow-up, 4 remained hospitalized and 7 (25%) had died ([Latif 2020](#)).

Preliminary data from Switzerland, however, were more hopeful ([Tschopp 2020](#)). Overall, 21 patients were included with a median

age of 56 years (10 kidney, 5 liver, 1 pancreas, 1 lung, 1 heart and 3 combined transplantations). Ninety-five percent and 24% of patients required hospital and ICU admission, respectively. After a median of 33 days of follow-up, 16 patients were discharged, 3 were still hospitalized and only 2 patients died.

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Other comorbidities

Ultimately, the current situation might lead to substantial changes in how research and medicine are practiced in the future. The SARS-CoV-2 pandemic has created major dilemmas in almost all areas of health care. Scheduled operations, numerous types of treatment and appointments have been cancelled worldwide or postponed to prioritise hospital beds and care for those

who are seriously ill with COVID-19. Throughout the world, health systems had to consider rapidly changing responses while relying on inadequate information. In some settings such as HIV or TB infection, oncology or solid organ transplantation, these collateral damages may have been even greater than the damage caused by COVID-19 itself. Treatment interruptions, disrupted drug supply chains and consequent shortages will likely exacerbate this issue. During the next months, we will learn more and provide more information on the consequences of this crisis on various diseases. In the meantime, please refer to the key papers listed below.

Oncology

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11. Pediatrics

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SARS-CoV-2 infection in children

Studies on the risk of acquiring SARS-CoV-2 infection in children in comparison to adults have shown contradicting results ([Mehta 2020](#), [Gudbjartsson 2020](#), [Bi 2020](#)). The exact role that children play in the transmission of SARS-CoV-2 is not yet fully understood. Population based studies performed so far indicate that children might not play a major factor in the spreading of COVID-19 ([Gudbjartsson 2020](#)). Seropravalence studies are lacking.

Children often have an asymptomatic or less severe COVID-19 disease course than adults ([Zimmermann 2020](#), [Parri 2020](#), [Ludvigsson 2020](#)). In this regard COVID is strikingly different from other virus-induced respiratory diseases, which can be fatal (e.g. RSV in infants). The CoV-2 pandemic causes a large collateral damage to children because they are taken out of their normal social environment (kindergartens, schools etc.), and because of parents anxiety to seek medical care despite need e.g. for vaccination ([Bramer 2020](#)) or even if their children are having an emergency ([Lazzerini 2020](#)).

Commonly circulating coronaviruses in children: tropism, incubation period and spreading

The first International Corona Virus Conference was organized by Volker Termeulen in Würzburg/Germany in 1980. At the time only one human coronavirus, HCoV2229E, was known to be associated with the common cold (Weiss 2020). Commonly circulating human coronaviruses can be isolated from 4-8% of all children with acute respiratory tract infections, which tend to be mild, unless the child is immunocompromised (Ogimi 2019). Seven coronaviruses circulate among humans: α -Coronaviruses HCoV2-229e, -HKU1; β -Coronaviruses HCoV2-NL63, -OC43; MERS-CoV, SARS-CoV and SARS-CoV-2 that have originally derived from bats (NL63, 229e, SARS-CoV), dromedary camels (229e, MERS-CoV), cattle (OC43), pangolins (SARS-CoV-2) (Zimmermann 2020). There appear to be re-infections with the earlier described common COV despite the fact that most individuals seroconvert to human coronaviruses. In many children there are co-infections with other viruses such as Adeno-, Boca-, Rhino-, RSV-, Influenza- or Parainfluenza virus. There seems to be a cyclic pattern with seasonal outbreaks between December and May or March to November in the southern hemisphere.

A characteristic of the single-strand RNA coronaviruses is the capability of rapid mutation and recombination leading to novel coronaviruses that can spread from animals to humans. They have caused epidemics leading to significant case fatality rates (10% in SARS-CoV, Hong Kong 2002; more than 30% in MERS-CoV, Saudi Arabia 2012). Because of the high case fatality rate, both SARS-COV and MERS-COV have a low potential for long-term sustained community transmission. Accordingly, no human SARS-CoV infections have been reported since July 2003.

It is estimated that in SARS-CoV-2 one person infects 2-3 other persons. In clusters (e.g. nosocomial outbreaks) this number might be much higher. In both SARS-CoV and MERS-CoV, super-

spreading events with one individual infecting up to 22 (SARS) or even 30 individuals (MERS) have been reported, especially in nosocomial outbreaks. In SARS-CoV a total of 41 children were reported with no deaths. Similarly, in MERS-CoV only 38 children were reported in two studies, with two deaths (Zimmermann 2020).

Epidemiology of COVID-19 in children

On April 6 the US CDC reported 2572 (1.7%) children under 18 years among 149,082 reported cases from 12 February to 2 April 2020. The availability of data was extremely limited (less than 10% available on symptoms, 13% on underlying conditions, 33% on whether children were hospitalized or not). Three deaths were reported to the CDC but no details were given. The median age was 11 and they were 57% males. 15 children were admitted to an ICU ($\leq 2\%$). Children <1 year accounted for the highest percentage (15–62%) of hospitalization (CDC 2020). The Chinese CDC report (Dong 2020) comprises 2,143 pediatric patients from January 16 to February 8 2020. Only 731 children (34.1%) were laboratory confirmed cases. The median age was 7 years with 56.6% boys, less than 5% were classified as severe and less than 1% as critical. One Chinese 10 month-old child who had been infected with CoV-2 was reported to have died with intussusception and multi-organ failure (Lu X 2020). The Korean Center for Disease Control and Prevention reported on 20 March that 6.3% of all COVID-19 cases were children under 19 years of age; again, the children had a mild form of the disease (Korean Center for Disease Control and Prevention. Press releases, <https://www.cdc.go.kr>). Italian data published on 18 March showed that only 1.2% of the 22,512 Italian cases with COVID-19 were children; no deaths were reported in this and in the Spanish cohort from Madrid (2 March to 16 March) (Livingstone 2020, Tagarro 2020).

The European Surveillance System (TESSy) collects data from EU/EEA countries and the UK on laboratory-confirmed cases of COVID-19. Out of 576,024 laboratory confirmed COVID-19 cases 0.7% were 0-4 years, 0.6% 5-9 years, 0.9% 10-14 years (<https://covid19-surveillance-report.ecdc.europa.eu>).

Natural course and risk factors for complications

The incubation period is believed to be 3-7 days (range 1-14 days) (She 2020), the clinical onset 5-8 days after infection with the virus. Children often have an asymptomatic or less severe COVID-19 disease course than adults (Zimmermann P 2020, Parri 2020). Among a total of 100 children with SARS-CoV-2 from Italy, 21% were asymptomatic, 58% had mild disease, 19% had moderate disease, 1% had severe disease, and 1% were in critical condition (Parri 2020).

At 10 days after onset of symptoms hyperinflammation may set in and cause a more severe and potentially fatal disease, especially in high risk groups. The clinical manifestation is believed to last for 1-2 weeks, longer in complicated cases. Due to the paucity of data it is as yet unclear which group of children may be at a higher risk for development of complications, e.g. children with underlying chronic pulmonary or cardiac disease, severe neurologic deficits, immunosuppressed or critically ill children etc. Analogous to influenza there might be genetic susceptibility in some children (Clohisey 2019). Interestingly, in a flash survey from 25 countries with 10,000 children with cancer at risk and 200 tested, only 9 were found to be CoV-2 positive. They were asymptomatic or had mild disease (Hrusak 2020).

In the European surveillance System (TESSy) deaths among children aged below 15 years are rare, 4 out of 44,695 (0.009%) were reported in the TESSy. The rate of hospitalization was higher in

children under the age of five especially in infants compared to persons aged 5-29. It is believed that the threshold for admission is lower in young children. A severe course requiring admission to ICU was not more likely in the younger children. The likelihood of being hospitalised was higher when children had an underlying condition, a severe course was rare (<https://covid19-surveillance-report.ecdc.europa.eu>).

In a cross-sectional study including 48 children with COVID-19 (median age 13 years; admitted to 46 North American pediatric ICUs between March 14 and April 3, 2020), forty patients (83%) had significant preexisting comorbidities and 18 (38%) required invasive ventilation. Targeted therapies were used in 28 patients (61%, mainly HCQ). Two patients (4%) died and 15 (31%) were still hospitalized, with 3 still requiring ventilatory support and 1 receiving ECMO (Shekerdemia 2020).

In an observational retrospective cohort study that included 177 children and young adults with clinical symptoms and laboratory confirmed SARS-CoV-2 infection treated between March 15 and April 30, 2020 at the Children's National Hospital in Washington, 44 were hospitalized and 9 were critically ill. Of these, 6/9 were adolescents and young adults > 15 years of age. Although asthma was the most prevalent underlying condition overall, it was not more common among patients with severe disease (DeBiasi 2020).

As of 11 May, 74 centers in Germany reported 137 pediatric hospital admissions, 15% were admitted to the ICU. 55.6% of the patients admitted to the ICU had an underlying disease, mostly pulmonary or cardiac diseases (www.dgpi.de).

Pathophysiology and immunopathology

It is unclear why COVID-19 in children is associated with a less severe disease course.

The tissue expression pattern of the receptor for CoV-2 angiotensin converting enzyme (ACE2) and the transmembrane serine protease TMPRSS2 (essential for CoV-2 cell entry) as well as the tissue tropism of CoV-2 in childhood are unknown. ACE2 is expressed on cells of the airways, the lungs, mucosal cells (lids, eyelids, nasal cavities), intestines and on immune cells (monocytes, lymphocytes, neutrophils) (Molloy 2020, reviewed in Brodin 2020). It needs to be clarified whether there is neurotropism (e.g. affecting the developing brain of newborns).

The main target of CoV-2 is the respiratory tract. As respiratory infections are extremely common in children it is to be expected that there are other viruses present in the respiratory tract of young children concomitantly with the coronavirus, which may limit its growth and the number of CoV-2 copies in the respiratory tract of children. Systematic viral load measurements in the respiratory tract of different viruses in children are underway. Key to the later immunopathologic stages of COVID-19 pneumonia is the macrophage activation syndrome (MAS)-like hyperinflammatory phase with a cytokine storm and acute respiratory distress syndrome ARDS, usually within 10-12 days after symptom onset. In general, children are not less prone to develop ARDS during respiratory tract infections than adults. In the H1N1 flu pandemic in 2009, being under the age of 1 year was a significant risk factor for developing a severe form of the infection and ARDS (Bautista 2010). Why ARDS is less common in children compared to adults with COVID-19 is unclear.

Regarding childhood immunity, an explanation for the milder disease course in children could be age-related differences in immune responses to CoV-2 between adults and children. To

what extent previous infections with non-SARS coronaviruses may have led to protective cross-reactive antibodies is unclear.

In the innate immune response damaged lung cells induce inflammation by macrophages and granulocytes. Based on influenza animal models it has been proposed that BCG vaccination (for tuberculosis prevention, done in the first week of life in some countries) may enhance non-specific innate immunity in children to infections like COVID-19 (so-called trained immunity) (Moorlag 2019). A search of the BCG World Atlas and correlation with data of COVID-19 cases and death per country found that countries without universal policies of BCG vaccination (Italy, the Netherlands, USA) have been more severely affected compared to countries with universal and long-standing BCG policies and that BCG vaccination also reduced the number of reported COVID-19 cases in a country (Miller 2020, Hauer 2020). Recent data from a large population-based study did not show decreased infection rates in Israeli adults aged 35 to 41 years who were BCG-vaccinated in childhood as compared to non-BCG-vaccinated. Data on the effect of BCG vaccination on COVID-19 disease severity are unavailable (Hamiel 2020).

In the adaptive response cytotoxic T cells play an important role in regulating responses to viral infections and control of viral replication. Children could benefit from the fact that the cytotoxic effector function of CD8 T cells in viral infection in children may be less detrimental compared to adults. Immune dysregulation with exhaustion of T cells has been reported in adults with COVID-19 infection. Regarding humoral immunity IgG maternal antibodies are actively transferred to the child via placenta and/or IgA via breast milk. They may not include anti CoV-2 antibodies, if the mother is naïve to CoV-2 or infected late in pregnancy. In mothers with COVID-19 pneumonia serum and throat swabs of their newborns were negative for CoV-2 but virus-specific IgG antibodies were detected (Zeng H 2020). Thus, neo-

nates may benefit from placental transmission of virus-specific antibodies from pre-exposed mothers. As shown in SARS CoV-1 it is likely that in SARS-CoV-2 a newly infected child will mount a significant humoral response with neutralizing IgM (within days) and IgG antibodies (within 1-3 weeks) to one of the immunodominant epitopes, e.g. the crown-like spike proteins giving the coronaviruses their name.

Data regarding IgG and IgM seroprevalence and quality of the immune response in children are lacking. No human reinfections with CoV-2 have been demonstrated yet but overall it is not clear whether children mount a durable memory immune response to CoV-2.

Transmission

Contraction of COVID-19 in a pregnant woman may have an impact on fetal outcome, namely fetal distress, potential preterm birth or respiratory distress if the mother gets very sick. As of yet there is no evidence that SARS-CoV-2 can be transmitted vertically from mother to child. Amniotic fluid, cord blood, neonatal throat swabs all tested negative in a small cohort (Chen 2020). Schwartz reviewed 5 publications from China and was able to identify 38 pregnant women with 39 offspring among whom 30 were tested for COVID-19 and all of them were negative (Schwartz 2020). Transmission by breastfeeding has not yet been reported and there are no case reports of detection of CoV-2 in breast milk.

SARS-CoV-2 in children is transmitted through family contacts and mainly through respiratory droplets (Garazzino 2020). In a study from France, child-to-child and child-to-adult transmission seems to be uncommon (Danis 2019). Prolonged exposure to high concentrations of aerosols may facilitate transmission (She 2020).

SARS-CoV-2 may also be transmitted through the digestive tract. ACE2 is also found in upper esophageal and epithelial cells as well as intestinal epithelial cells in the ileum and colon (She 2020). SARS-CoV-2 RNA can be detected in the feces of patients (Holshue 2020). Cai revealed that viral RNA is detected from feces of children at a high rate (and can be excreted for as long as 2-4 weeks) (Cai 2020). However, direct evidence of a fecal-to-oral transmission has not yet been documented.

Diagnosis and classification

Testing for the virus is only necessary in clinically suspect children. If the result is initially negative, repeat nasopharyngeal or throat swab-testing of upper respiratory tract samples or testing of lower respiratory tract samples should be done. Sampling of the lower respiratory tract (induced sputum or bronchoalveolar lavage) is more sensitive (Han 2020). This is not always possible in critically ill patients and in young children.

Diagnosis is usually made by real-time polymerase chain reaction RT-PCR on respiratory secretions. For SARS-CoV, MERS-CoV and SARS-CoV-2, higher viral loads have been detected in samples from lower respiratory tract compared with upper respiratory tract.

In some patients, SARS-CoV-2 RNA is negative in respiratory samples while stool samples are still positive indicating that the viral gastrointestinal infection can last even after viral clearance in the respiratory tract. (Xiao 2020). Fecal testing may thus be of value in diagnosing CoVID-19 in these patients.

As in other viral infections, a CoV-2 IgM and IgG seroconversion will appear in days (IgM) to 1-3 weeks (IgG) after infection and may or may not indicate protective immunity (still to be determined). Interestingly, asymptomatic seroconversion has been hypothesized in a very small series of health workers (mean age

40 years) exposed to a child with COVID-19 in a pediatric dialysis unit (Hains 2020).

Serology may be useful in patients with clinical symptoms highly suggestive of SARS-CoV-2 who are RNA negative, i.e in children with pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2 (PIMS-TS). In case serology indicates protective immunity, this will be extremely important from a public health perspective, e.g. it will allow for strategic staffing in medical care and for the assessment of CoV-2 epidemiology (herd immunity).

Table 1. COVID classification in children (Shen 2020)

1	Asymptomatic without any clinical symptoms
2	Mild fever, fatigue, myalgia and symptoms of acute respiratory tract infections,
3	Moderate pneumonia, fever and cough, productive cough, wheezing but no hypoxemia
4	Severe fever, cough, tachypnea, oxygen saturation less than 92%, somnolence
5	Critical quick progress to acute respiratory distress syndrome ARDS or respiratory failure

Laboratory and radiology findings

Laboratory and/or radiology studies in outpatient children who have mild disease are not indicated. Upon admission to the hospital the white blood cell count is usually normal. In a minority of children decreased lymphocyte counts have been documented. In contrast, adults (with hyperinflammation and cytokine release syndrome) often have an increase in neutrophils and lymphopenia. The inflammation parameters C-reactive protein and procalcitonin can be slightly elevated or normal while there are elevated liver enzymes, creatine kinase CK-MB and D-dimers

in some patients. LDH appears to be elevated in severe cases and can be used to monitor severe disease.

A chest X-ray should only be done in children with moderate or more severe disease as CT scans mean a very high radiation exposure for the child and should only be done in complicated or high-risk cases. In the beginning of the pandemic in China, children all received CT scans even when they were asymptomatic and oligosymptomatic; surprisingly, they displayed very severe changes. On chest radiography there are bilateral patchy air-space consolidations and so-called ground-glass opacities. CT scans were more impressive than chest x-ray examinations. In 20 children with CT, 16 (80%) had some abnormalities (Xia 2020).

Symptoms and signs: Acute infection

Children and adolescents

The clinical presentation of the disease appears somewhat similar to influenza. In the largest clinical trial of 171 children from Wuhan fever was reported in 41% (71 of 171), cough in over 50% (83 of 171), tachypnea in 28% (49 of 171). In 27 of the patients there were no symptoms at all (15.8%). At initial presentation very few children required oxygen supplementation (4 of 171, 2.3%). Other symptoms like diarrhea, fatigue, runny nose and vomiting were observed only in less than 10% of the children (Lu 2020). In the case series from Zhejiang as many as 10 out of 36 patients (28%) had no symptoms at all. None of the children had an oxygen saturation below 92% (Qiu 2020).

Neonates and infants

Zeng reports 33 newborns born to mothers with COVID-19 in Wuhan. Three of the 33 infants (9%) presented with early-onset SARS-CoV-2 infection. In 2 of the 3 neonates there were radiological signs of pneumonia. In one child disseminated intravascular

coagulation was described but eventually all children had stable vital signs three weeks after the infection when the report was published (26 March 2020) (Zeng L 020). In a second cohort, 9 infants aged 1 month to 9 months were described without any severe complications (Wei 2020). Whether there may be complications of COVID-19 in newborns and infants long-term cannot be judged at this stage of the pandemic. At present it is not recommended to separate healthy newborns from mothers with suspicion of COVID-19 (CDC-2 2020). Clearly a preterm or newborn that has been exposed to CoV-2 needs to be closely monitored by the hospital and/or the primary care pediatrician. If there are signs of COVID (e.g. poor feeding, unstable temperature, tachy/dyspnea) it needs to be hospitalized and tested and lab examinations and chest x-ray to be done. Testing for CoV-2 is not useful before day 5 because of the incubation period. There needs to be a strict hygiene as much as possible in this mother-child setting.

Pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2 (PIMS-TS) (or synonym Multi-system Inflammatory Syndrome in Children (MIS-C) or Kawasaki-like Disease

In April 2020 clinicians from the UK, France, Italy, Spain and the US reported on children with a severe inflammatory syndrome with Kawasaki-like features, some of whom had tested positive for CoV-2, while others had not. Prior to this, Jones had described the case of a six-month-old baby girl with fever, rash and swelling characteristic of a rare pediatric inflammatory condition, Kawasaki syndrome (Jones 2020).

Eight patients from the UK and 10 patients from Bergamo in Italy with features of Kawasaki disease have been published including one death in a 14-year-old boy in the UK during the SARS-CoV-2 epidemic (Riphagen 2020, Verdoni 2020). In Bergamo, the region

with the highest infection rate in Italy, a 30-fold increased incidence of Kawasaki disease has been reported following the SARS-CoV-2 epidemic (Verdoni 2020).

Currently, the pathophysiological overlap between COVID-19 associated inflammation and Kawasaki disease is not yet clear, their features are summarized in Table 2. asdf

Table 2. Features of Kawasaki Disease and pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2

	Kawasaki (Hedrich 2017, ECDC 2020) (previously called mucocutaneous lymph-node syndrome)	PIMS-TS (pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2 or MIS-C (multisystem inflammatory syndrome in children) (Verdoni 2020; Riphagen 2020, https://covid19-surveillance-report.ecdc.europa.eu/) “ Kawasaki-like disease ”
Epidemiology	Incidence 5–19/100,000 annually < 5 years of age (EU, US), in north-east Asia higher; seasonal increase in winter/spring, geographic wave-like spread of illness during epidemics (Rowley 2018)	Incidence unknown. 230 suspected cases temporally associated with COVID-19 reported to ECDC by May 15 th (EU/EEA, UK). More common in afro-caribbean descent, obesity? (Riphagen 2020)
Age, sex	>90% < 5 years of age, more males	5-15 years of age, sex distribution unclear

Table 2. Features of Kawasaki Disease and pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2

Etiology	Unknown, hypothesis: infection with common pathogens, e.g. bacteria, fungi and viruses which cause immune-mediated damage (Dietz 2017) (Jordan-Villegas 2010 , Kim 2012 , Turnier 2015). Genetic factors (increased frequency in Asia and among family members of an index case)	Unknown, no working hypothesis yet. Hyperinflammation/shock associates with immune response to SARS-CoV-2. In CoV-1 antibody-dependent enhancement (ADE): presence of antibodies can be detrimental, enable the virus to spread (demonstrated in SARS-CoV)
Case definition	fever ≥ 5 days, combined with at least 4 of the 5 following items 1. Bilateral bulbar conjunctival injection 2. Oral mucous membrane changes, including injected or fissured lips, injected pharynx, or strawberry tongue 3. Peripheral extremity changes, including erythema of palms or soles, edema of hands or feet (acute phase) or periungual desquamation (convalescent phase) 4. Polymorphous rash 5. Cervical lymphadenopathy (McCrindle 2017) Children suspected of having KD who do not fulfill diagnostic criteria may have incomplete or atypical KD (Cimaz 2009)	1. Persistent fever, inflammation (neutrophilia, elevated CRP and lymphopenia) and single or multi-organ dysfunction (shock, cardiac, respiratory, renal, gastrointestinal or neurological disorder) with other additional clinical, laboratory or imaging and ECG features. Children fulfilling full or partial criteria for Kawasaki disease may be included 2. Exclusion of any other microbial cause, including bacterial sepsis, staphylococcal or streptococcal shock syndromes, infections associated with myocarditis such as enterovirus 3. SARS-CoV-2 PCR testing positive or negative. (Royal College of Paediatrics and Child Health)

Table 2. Features of Kawasaki Disease and pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2

CoV-2 status in most cases	CoV-2 Ag (PCR); Abs (Elisa) negative	CoV-2 Ag (PCR) negative and Abs (Elisa) positive
Typical Lab	Marked Elevation of acute-phase reactants (eg, C-reactive protein [CRP] or erythrocyte sedimentation rate [ESR]) Thrombocytosis (generally after day 7 of illness) Leukocytosis, left-shift (increased immature neutrophils)	Marked elevation of acute phase reactants CRP, ESR Thrombocytopenia Leucopenia Lymphopenia Hyperferritinemia Elevated myocarditis markers Troponin, pro-BNP
Acute Complications	Kawasaki disease shock syndrome (KSSS) (rare), features of macrophage activation syndrom, MAS (rare), coronary artery abnormalities, mitral regurgitation, prolonged myocardial dysfunction, disseminated intravascular coagulation (Kanegaye 2009) Gastrointestinal complications (Ileitis, vomiting, abdominal pain) rare	Shock (common), features of macrophage activation syndrome (common), myocardial involvement evidenced by markedly elevated cardiac enzymes (common), myocardial infarction, aneurysms, disseminated intravascular coagulation Gastrointestinal complications (Ileitis, vomiting, abdominal pain) are very common
Long term Complications	Artery abnormalities (aneurysms of mid-sized arteries, giant coronary artery aneurysms CAAs)	Unknown; aneurysms?

Table 2. Features of Kawasaki Disease and pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2

Management	High-dose intravenous immunoglobulin (IVIG) (2g/kg) first-line treatment; effective in reducing the risk of coronary artery disease when administered within 10 days of onset of fever. In addition, acetylsalicylic acid, glucocorticoids and anti-TNF monoclonal antibodies have been used	So far, most patients published were treated with high dose IVIG, glucocorticoids, ASS (Verdoni 2020, Riphagen 2020) IVIG resistance requiring adjunctive steroid treatment is common (Verdoni 2020.) Management on the pediatric intensive care unit is often necessary: progression to vasoplegic shock is common Hemodynamic support, treatment with noradrenaline and milrinone, mechanical ventilation is often required (Riphagen 2020)
Prognosis	Self-limited vasculitis lasting for an average of 12 days without therapy. Without timely treatment, CAAs, and in particular aneurysms, can occur in up to 25% of children	Overall prognosis not yet clear More severe course than KD Potentially fatal in individual cases

Management

Infection control

Early identification of COVID-19 and quarantine of contacts is imperative. In the in- and out-patient setting it is advised to separate children who have infectious diseases from healthy non-infectious children. Nosocomial outbreaks have played a role in the clustering of COVID-19. Thus it is advised to admit children with COVID-19 to the hospital only if an experienced pediatrician feels it is medically necessary (e.g. tachypnea, dyspnea, oxygen levels below 92%). In the hospital the child with COVID-19

or suspicion of COVID-19 needs to be isolated in a single room or admitted to a COVID-19-only ward in which COVID-19 exposed medical personnel maintains distance as well (e.g. no shifts on other wards). The presence of one parent is not negotiable in the care of the sick child both for emotional reasons as well as for help in nursing of the child.

During the peak phase of the COVID-19 epidemic, precautions in the outpatient and hospital setting include entrance control, strict hand and respiratory hygiene, daily cleaning and disinfection of the environment, and provision of protection (gloves, mask, goggles) for all medical staff when taking care of a COVID-19 or a suspected COVID-19 case (Wang 2020). In neonatal intensive care units (NICU), negative pressure rooms and filtering of exhaust would be ideal (Lu Q 2020). Respirators with closed circuit and filter systems should be used. Aerosol generating procedures, e.g. intubation, bronchoscopy, humidified inhalations/nebulization should be avoided as much as possible.

Supportive treatment (respiratory support, bronchodilatation therapy, fever, superinfection, psychosocial support)

Having the child sitting in an upright position will be helpful for breathing. It might be useful to have physiotherapy. Insufflation of oxygen via nasal cannula will be important to children as it will increase lung ventilation and perfusion. In neonates, high flow nasal cannula (HFNC) has been utilized widely due to its superiority over other non-invasive respiratory support techniques.

The clinical use and safety of inhaling different substances in COVID-19 is unclear. In other common obstructive and infectious childhood lung diseases, e.g. in bronchiolitis, the American Academy of Pediatrics is now recommending against the use of

bronchodilators (Dunn 2020). Regarding the inhalation of steroids as part of maintenance therapy for asthma bronchiale there is no evidence to discontinue this treatment in children with COVID-19.

There is a large controversy over the extent of antipyretics usage in children. Still, in a child with COVID-19 who is clinically affected by high-degree fever, paracetamol or ibuprofen may be useful. There is no restriction despite initial WHO warnings of using ibuprofen, there is no evidence that the use of paracetamol or ibuprofen is harmful in COVID-19 in children (Day 2020).

The differentiation between CoV-2-induced viral pneumonia and bacterial superinfection is difficult unless there is clear evidence from culture results or typical radiological findings. Bacterial superinfection will be treated according the international and national guidelines (Mathur 2018).

The virus outbreak brings psychological stress to the parents and family as well as medical staff; therefore, social workers and psychologists should be involved when available.

Treatment of respiratory failure

The treatment of pediatric acute respiratory distress syndrome (pARDS) is reviewed elsewhere (Allareddy 2019). For neonates with pARDS high-dose pulmonary surfactant replacement, nitric oxide inhalation, and high-frequency oscillatory ventilation might be effective. In critically ill neonates, continuous renal replacement and extracorporeal membrane oxygenation need to be implemented if necessary.

COVID-19-specific drug treatment

As of yet there are no data from controlled clinical trials and thus there is currently no high-quality evidence available to support the use of any medication to treat COVID-19. The drugs

listed below are repurposed drugs and there is limited or almost no pediatric experience. In the case of a severe or critically ill child with COVID the pediatrician has to make a decision whether to try a drug or not. If initiation of a drug treatment is decided, children should be included into clinical trials (<https://www.clinicaltrialsregister.eu>) if anyhow possible. However, there are only very few, if any, studies open for recruitment in children.

When to treat with drugs

Under the lead of the German Society for Pediatric Infectiology (DGPI) an expert panel has proposed a consensus on when to start antiviral or immunomodulatory treatment in children (Table 3, <https://dgpi.de/stellungnahme-medikamentoes-behandlung-von-kindern-mit-covid-19/>).

A panel of pediatric infectious diseases physicians and pharmacists from North American institutions published an initial guidance on use of antivirals for children with SARS. It is advised to limit antiviral therapy to children in whom the possibility for benefit outweighs risk of toxicity and remdesvir is the preferred agent (Chiotos 2020).

Inhibitors of viral RNA synthesis

Remdesivir (GS-5734) is available as 150 mg vials. Child dosing is

- <40 kg: 5 mg/kg iv loading dose, then 2.5 mg/kg iv QD for 9 days
- ≥40 kg: 200 mg loading dose, then 100 mg QD for 9 days

Remdesivir is an adenosine nucleotide analogue with broad-spectrum antiviral activity against various RNA viruses. The compound undergoes a metabolic mechanism, activating nucleoside triphosphate metabolites for inhibiting viral RNA polymer-

ases. Remdesivir has demonstrated *in vitro* and *in vivo* activity in animal models against MERS and SARS-CoV. Remdesivir showed good tolerability and a potential positive effect in regard to decrease of the viral load and mortality in Ebola in Congo in 2018 (Mulangu 2019). In Europe this drug has rarely been used in children so one should be extremely careful. It can be obtained through compassionate use programs (<https://rdvcu.gilead.com>)

Table 3. Consensus on antiviral or immunomodulatory treatment in children

Disease severity in child	Intervention
Mild or moderate disease pCAP, upper respiratory tract infection, no need for oxygen	Treat symptomatically. No need for antiviral or immunomodulatory treatment
More severe disease and risk groups* pCAP, need for oxygen	Treat symptomatically consider antiviral therapy
Critically ill, admitted to ICU	Treat symptomatically. Consider antiviral therapy. Consider immunomodulatory treatment
Secondary HLH (hemophagocytic lymphohistiocytosis)	Treat with immunomodulatory or immunosuppressive drugs

* Congenital heart disease, immunosuppression, inborn/acquired immunodeficiencies, cystic fibrosis, chronic lung disease, chronic neurological/kidney/liver disease, diabetes/metabolic disease

Lopinavir/r (LPV/r, Kaletra®) is a co-formulation of lopinavir and ritonavir, in which ritonavir acts as a pharmacokinetic enhancer (booster). LPV/r is an HIV-1 protease inhibitor successfully used in HIV-infected children as part of highly active antiretroviral combination therapy (PENTA Group, 2015). In the SARS epidemics, LPV/r had been recommended as a treatment. A recent study in adult COVID-19 patients did not show an effect

regarding the primary end point in a controlled clinical trial. Despite the fact that there is a large experience with LPV/r in HIV, it is not advised to use it in children with COVID-19 as it does not appear to be effective at all (see Treatment chapter, page 233).

Inhibitors of viral entry

Hydroxychloroquine (HCQ, Quensyl®), Chloroquine (CQ, Resochin junior®, Resochin®) The experience among pediatricians with HCQ/CQ (except pediatricians working with malaria) is very limited. Authorities in the US are now warning about a widespread use of HCQ/CQ in COVID-19 (<https://mailchi.mp/clintox/aact-acmt-aapcc-joint-statement>). It is not advised to use HCQ or CQ in children with COVID as neither drug appears to be effective at all (see chapter Treatment, page 233).

Immunomodulatory drug treatment

The rationale for immunomodulation in COVID-19 patients comes from a high expression of pro-inflammatory cytokines (Interleukin-1 (IL1) and interleukin-6 (IL6)), chemokines (“cytokine storm”) and the consumption of regulatory T cells resulting in damage of the lung tissue as reported in patients with a poor outcome. **Blocking IL-1 or IL-6** can be successful in children with (auto) inflammatory disease (reviewed in Niehues 2019). However, both interleukins are also key to the physiological immune response and severe side effects of immunomodulators have been reported. In adults with COVID-19, blocking interleukin-1/6 might be helpful (see the Treatment chapter). In the rare situation that the condition of the child deteriorates due to hyperinflammation and that they are resistant to other therapies, tocilizumab or anakinra may be an option.

Steroids (e.g. prednisone, prednisolone) are available as oral solution, tablets or different vials for intravenous application.

Dosage in children is 0.5 to 1 mg/kg i.v. or oral BID. Short term use of steroids has few adverse events. Administration of steroids will affect inflammation by inhibiting the transcription of some of the pro-inflammatory cytokines and various other effects. The use of corticosteroids in children and adults with CoV-induced ARDS is controversial (Lee 2004, Arabi 2018, Russell 2020). The corticosteroid-induced decrease of antiviral immunity (e.g. to eliminate CoV-2 viruses) might be disadvantageous in patients with COVID-19. The use of low-dose hydrocortisone may be of advantage in adults with ARDS, whereas its use is controversial in pediatric ARDS. Most patients with pediatric inflammatory multisystem syndrome associated with SARS-CoV-2 (PIMS-TS) published so far were treated with high dose IVIG and methylprednisolone (Verdoni 2020, Riphagen 2020). In these patients, features of macrophage activation syndrome and IVIG resistance were common, requiring adjunctive steroid treatment (Verdoni 2020).

Tocilizumab (Roactemra®) is available in 80/200/400 mg vials (20 mg/ml). Dosing is

- <30 kg: 12 mg/kg iv QD, sometimes repeated after 8 hrs
- ≥30 kg: 8mg/kg iv QD iv (max. 800 mg)

Adverse events (deriving largely from long term use in chronic inflammatory diseases and use in combination with other immunomodulatory drugs): severe bacterial or opportunistic infections, immune dysregulation (anaphylactic reaction, fatal macrophage activation), psoriasis, vasculitis, pneumothorax, fatal pulmonary hypertension, heart failure, gastrointestinal bleeding, diverticulitis, gastrointestinal perforation (reviewed in Niehues 2019).

Anakinra (Kineret®) is available as 100 mg syringes (stored at 4-8° C). Dosing is 2-4 mg/kg s.c. QD daily as long as hyperinflammation persists. Thereafter, dose reduction by 10-30% per day.

Adverse events (deriving largely from long-term use in chronic inflammatory diseases and use in combination with other immunomodulatory drugs): severe bacterial or opportunistic Infections, fatal myocarditis, immune dysregulation, pneumonitis, colitis, hepatitis, endocrinopathies, nephritis, dermatitis, encephalitis, psoriasis, vitiligo, neutropenia (reviewed in Niehues 2019).

Immunotherapy

Engineering **monoclonal antibodies** against the CoV spike proteins or against its receptor ACE2 or **specific neutralizing antibodies** against CoV-2 present in convalescent plasma may provide protection but are not generally available yet.

Interferon α has been inhaled by children with COVID-19 in the original cohorts but there are no data on its effect (Qiu 2020). Type-1 interferons (e.g. interferon- α) are central to antiviral immunity. When coronaviruses (or other viruses) invade the host, viral nucleic acid activates interferon-regulating factors like IRF3 and IRF7 which promote the synthesis of type I interferons (IFNs).

PIMS / MIS-C / Kawasaki-like disease

Based on the information published so far, most patients were treated with high dose intravenous Immunglobulin (see Table 2) and corticosteroids (Verdoni 2020). More data are needed to determine the optimal treatment strategies for patients with MIS-C.

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12. Timeline

Sunday, 1 December

According to a retrospective study published in *The Lancet* on 24 January 2020², the earliest laboratory confirmed case of COVID-19 in Wuhan was in a man whose symptoms began on 1 December 2019. No epidemiological link could be found with other early cases. None of his family became ill.

Thursday, 12 December

In **Wuhan**, health officials start investigating a cluster of patients with viral pneumonia. They eventually find that most patients have visits to the Huanan Seafood Wholesale Market in common. The market is known for being a sales hub for poultry, bats, snakes, and other wildlife.

Monday, 30 December 2019

Li Wenliang (en.wikipedia.org/wiki/Li_Wenliang), a 34-year-old ophthalmologist from Wuhan, posts a message on a WeChat group alerting fellow doctors to a new disease at his hospital in late December. He writes that seven patients have symptoms similar to SARS and are in quarantine. Li asks his friends to inform their families and advises his colleagues to wear protective equipment.

² Huang, Chaolin et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China January 24, 2020 [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(20\)30183-5/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30183-5/fulltext)

Tuesday, 31 December 2019

The Wuhan police announce that they are investigating eight people for spreading rumors about a new infectious diseases outbreak (see 30 December).

The Wuhan Municipal Health Commission [reports 27 patients](#) with viral pneumonia and a history of exposure to the Huanan Seafood Wholesale Market. Seven patients are critically ill. The clinical manifestations of the cases were mainly **fever**, a few patients had **difficulty breathing**, and chest radiographs showed **bilateral lung infiltrative lesions**. The report says that the “disease is preventable and controllable”. WHO is informed about the outbreak.

Thursday, 1 January

The Huanan Seafood Wholesale Market is shut down.

Friday, 3 January

While examining bronchoalveolar lavage fluid collected from hospital patients between 24 and 29 December, Chinese scientists at the National Institute of Viral Disease Control and Prevention ruled out the infection with 26 common respiratory viruses, determined the genetic sequence of a novel β -genus coronavirus (naming it '2019-nCoV') and identified three distinct strains.³

Li Wenliang is summoned to a local public security office in Wuhan for “spreading false rumours”. He is forced to sign a docu-

³ Notes from the Field: An Outbreak of NCIP (2019-nCoV) Infection in China — Wuhan, Hubei Province, 2019–2020, China CDC Weekly, 2020, 2(5): 79–80
<http://weekly.chinacdc.cn/en/article/id/e3c63ca9-dedb-4fb6-9c1c-d057adb77b57>

ment where he admits having made “false comments” and “disrupted social order.” Li signs a statement agreeing not to discuss the disease further.

On the Weibo social network, Wuhan police say they have taken legal action against people who “published and shared rumors online”, “causing a negative impact on society”. The following day, the information is taken up by CCTV, the state television. CCTV does not specify that the eight people accused of “spreading false rumors” are doctors.

Sunday, 5 January

WHO issues an alert that 44 patients with pneumonia of unknown etiology have been reported by the national authorities in China. Of the 44 cases reported, 11 are severely ill while the remaining 33 patients are in stable condition.
<https://www.who.int/csr/don/05-january-2020-pneumonia-of-unknown-cause-china/en/>

Tuesday, 7 January

Chinese officials announce that they have identified a **new coronavirus** (CoV) from patients in Wuhan (pre-published 17 days later: <https://doi.org/10.1056/NEJMoa2001017>). Coronaviruses are a group of viruses that cause diseases in mammals and birds. In humans, the most common coronaviruses (HCoV-229E, -NL63, -OC43, and -HKU1) continuously circulate in the human population; they cause colds, sometimes associated with fever and sore throat, primarily in the winter and early spring seasons. Two coronavirus have also been responsible for human outbreaks of SARS and MERS. These viruses are spread by inhaling droplets generated when infected people cough or sneeze, or by touching a surface where these droplets land and then touching one's face.

Friday, 10 January

The gene sequencing data of the new virus was posted on Virological.org by researchers from Fudan University, Shanghai. A further three sequences were posted to the Global Initiative on Sharing All Influenza Data (GISAID) [portal](#).

On 10 January 2020, Li Wenliang, coronavirus whistleblower, started having symptoms of a dry cough. Two days later, Wenliang started having a fever and was admitted to the hospital on 14 January 2020. His parents also contracted the coronavirus and were admitted to the hospital with him. Wenliang tested negative several times until finally testing positive for the coronavirus on 30 January 2020.

Sunday, 12 January

Using the genetic sequence of the new coronavirus made available to WHO, laboratories in different countries start producing specific **diagnostic PCR tests**.

The Chinese government reports that there is no clear evidence that the virus passes easily from person to person.

Monday, 13 January

Thailand reports the first case outside of China, a woman who had arrived from Wuhan. Japan, Nepal, France, Australia, Malaysia, Singapore, South Korea, Vietnam, Taiwan, and South Korea report cases over the following 10 days.

Tuesday, 14 January

WHO tweeted that “preliminary investigations conducted by the Chinese authorities have found no clear evidence of human-to-human transmission of the novel coronavirus (2019-nCoV) identified in Wuhan, China”. On the same day, WHO’s Maria Van Kerkhove said that there had been “limited human-to-human

transmission” of the coronavirus, mainly small clusters in families, adding that “it is very clear right now that we have no sustained human-to-human transmission”⁴

Saturday, 18 January

The Medical Literature Guide **Amedeo** (www.amedeo.com) draws the attention of 50,000+ subscribers to a study from Imperial College London, *Estimating the potential total number of novel Coronavirus cases in Wuhan City, China*, by Imai et al. The authors estimate that “a total of 1,723 cases of 2019-nCoV in Wuhan City (95% CI: 427 – 4,471) had onset of symptoms by 12th January 2020”. Officially, only 41 cases were reported by 16th January.

Monday, 20 January

China reports three deaths and more than 200 infections. Cases are now also diagnosed outside Hubei province (Beijing, Shanghai and Shenzhen). Asian countries begin to introduce mandatory screenings at airports of all arrivals from high-risk areas of China.

After two medical staff were infected in Guangdong, the investigation team from China's National Health Commission confirmed for the first time that the coronavirus can be transmitted between humans.⁵

4 WHO says new China coronavirus could spread, warns hospitals worldwide". Reuters. 14 January 2020.

5 <https://www.theguardian.com/world/2020/jan/20/coronavirus-spreads-to-beijing-as-china-confirms-new-cases>

Wednesday, 22 January 2020

A WHO China office field mission to Wuhan issued a statement saying that there was evidence of human-to-human transmission in Wuhan, but more investigation was needed to understand the full extent of transmission.⁶

Thursday, 23 January

In a bold and unprecedented move, the Chinese government puts tens of millions of people in **quarantine**. Nothing comparable has ever been done in human history. Nobody knows how efficient it will be.

All events for the Lunar New Year (starting on January 25) are cancelled.

The WHO IHR (2005) Emergency Committee convened on 22-23 January acknowledged that human-to-human transmission was occurring with a preliminary R_0 estimate of 1.4-2.5 and that 25% of confirmed cases were reported to be severe. However, the Committee felt that transmission was limited and there was “no evidence” of the virus spreading at community level outside of China. Since the members could not reach a consensus, the committee decided that it was still too early to declare a Public Health Emergency of International Concern (PHEIC) and agreed to reconvene in approximately ten days’ time.⁷

A scientific preprint from the Wuhan institute of Virology, later published in *Nature*, announced that a bat virus with 96% similarity had been sequenced in a Yunnan cave in 2013. The sequence

⁶ <https://www.who.int/china/news/detail/22-01-2020-field-visit-wuhan-china-jan-2020>

⁷ [https://www.who.int/news-room/detail/23-01-2020-statement-on-the-meeting-of-the-international-health-regulations-\(2005\)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-\(2019-ncov\)](https://www.who.int/news-room/detail/23-01-2020-statement-on-the-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov))

is posted the next day on public databases.⁸ It is confirmed that the novel coronavirus uses this same entry receptor as SARS-CoV.

Friday, 24 January

At least 830 cases have been diagnosed in nine countries: China, Japan, Thailand, South Korea, Singapore, Vietnam, Taiwan, Nepal, and the United States.

The first confirmed evidence of human-to-human transmission outside of China was documented by the WHO in Vietnam.⁹

France reported its first three confirmed imported cases, the first occurrences in the EU.¹⁰

Zhu et al. publish their comprehensive report about the isolation of a **novel coronavirus** which is different from both MERS-CoV and SARS-CoV (full-text: <https://doi.org/10.1056/NEJMoa2001017>). They describe sensitive assays to detect viral RNA in clinical specimens.

Huang et al. publish on *The Lancet* the **clinical features** of 41 patients (full-text: [doi.org/10.1016/S0140-6736\(20\)30185-9](https://doi.org/10.1016/S0140-6736(20)30185-9)). The report indicated the risk of contagious infection without forewarning signs during the incubation period and suggested a “pandemic potential” for the new virus.

8 Zhou, Peng et al. "A pneumonia outbreak associated with a new coronavirus of probable bat origin". *Nature*. 579 (7798): 270–273
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7095418/>

9 "Novel Coronavirus (2019-nCoV) SITUATION REPORT - 4" WHO 24 January 2020.

10 "Coronavirus : un troisième cas d'infection confirmé en France". *Le Monde.fr* (in French). 24 January 2020.

Chan et al. describe a **familial cluster** of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission (full-text: [doi.org/10.1016/S0140-6736\(20\)30154-9](https://doi.org/10.1016/S0140-6736(20)30154-9)).

Saturday, 25 January

The Chinese government imposes travel restrictions on more cities in Hubei. The number of people affected by the quarantine totals **56 million**.

Hong Kong declares an emergency. New Year celebrations are cancelled and links to mainland China restricted.

Monday, 27 January

In Germany, the first cluster of infections with person to person transmission from asymptomatic patients in Europe **was reported**. The source of infection was an individual from Shanghai visiting a company in Bavaria¹¹. She developed symptoms on the way back to China. Contacts at the company were tested and transmission was confirmed to asymptomatic contacts but also to people who had no direct contact with the index patient. Authors state that “The fact that asymptomatic persons are potential sources of 2019-nCoV infection may warrant a reassessment of transmission dynamics of the current outbreak.”¹²

¹¹ Böhmer MM, Buchholz U, Cormann VM: **Investigation of a COVID-19 outbreak in Germany resulting from a single travel-associated primary case: a case series**. Published online May 15, 2020. Full-text: [https://www.thelancet.com/journals/laninf/article/PIIS1473-3099\(20\)30314-5/fulltext](https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30314-5/fulltext)

¹² Rothe C, Schunk M, Sothmann P, et al. **Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany**. N Engl J Med 2020;382:970-971. <https://pubmed.gov/32003551>. Full-text: <https://doi.org/10.1056/NEJMc2001468>

Tuesday, 28 January

WHO DG Dr. Tedros Adhanom Ghebreyesus met China President Xi Jinping in Beijing. They shared the latest information on the outbreak and reiterated their commitment to bring it under control. The WHO delegation highly appreciated the actions China has implemented in response to the outbreak, its speed in identifying the virus and openness to sharing information with WHO and other countries.¹³

Thursday, 30 January

On the advice of the IHR Emergency Committee, WHO DG declared a Public Health Emergency of International Concern and advised “all countries should be prepared for containment, including active surveillance, early detection, isolation and case management, contact tracing and prevention of onward spread of 2019-nCoV infection, and to share full data with WHO.” WHO had received reports of 83 cases in 18 countries outside China and that there had been evidence of human-to-human transmission in 3 countries.

China reports 7,711 cases and 170 deaths. The virus has now spread to all Chinese provinces.

Giuseppe Conte, Italy’s Prime Minister, confirms the first two COVID-19 imported cases in Italy.

Friday, 31 January

Li Wenliang publishes his experience with **Wuhan police station** (see 3 January) with the letter of admonition on social media. His post goes viral.

¹³ <https://www.who.int/news-room/detail/28-01-2020-who-china-leaders-discuss-next-steps-in-battle-against-coronavirus-outbreak>

India, the Philippines, Russia, Spain, Sweden, the United Kingdom, Australia, Canada, Japan, Singapore, the US, the UAE and Vietnam confirm their first cases.

Sunday, 2 February

The first death outside China, of a Chinese man from Wuhan, is reported in the **Philippines**. Two days later a death in Hong Kong is reported.

Thursday, 6 February

Li Wenliang, who was punished for trying to raise the alarm about coronavirus, dies. His death sparks an explosion of anger, grief and demands for freedom of speech: <https://www.theguardian.com/global-development/2020/feb/07/coronavirus-chinese-rage-death-whistleblower-doctor-li-wenliang>.

Friday, 7 February

Hong Kong introduces **prison sentences** for anyone breaching quarantine rules.

Saturday, 8 February

The French Health Minister confirmed that a cluster of 5 COVID-19 cases were detected in a ski resort in the French Alps. The index patient was a UK citizen who had traveled to Singapore on 20-23 January and then spent four days (24-28 January) in a chalet in Contamines-Montjoie, in Haute-Savoie. He tested positive upon return to England. Four contacts in the same chalet tested positive, including a 9-year old boy who was attending a local school. None of the child's contacts in school or at home became infected.

Monday, 10 February

Amedeo launches a weekly Coronavirus literature service which would later be called *Amedeo COVID-19*.

Tuesday, 11 February

Less than three weeks after introducing mass quarantine measures in China, the number of daily **reported cases starts dropping**.

The WHO announces that the new infectious disease would be called **COVID-19 (Coronavirus disease 2019)** and that the new virus will be called SARS-CoV-2.

Wednesday, 12 February

On board the Diamond Princess **cruise ship** docked in Yokohama, Japan, 175 people are infected with the virus. Over the following days and weeks, almost 700 people will be infected onboard.

Thursday, 13 February

China changed the COVID-19 case definition to include clinical (radiological) diagnosis of patients without confirmatory test. As a result, Hubei reported 14,840 newly confirmed cases, nearly 10 times more than the previous day, while deaths more than doubled to 242. WHO indicated that for consistency it would report only the number of laboratory-confirmed cases.¹⁴

¹⁴ <https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200213-sitrep-24-covid-19.pdf>

Wednesday, 19 February

Iran reports two deaths from the coronavirus.

At the San Siro stadium in Milan, the Atalanta soccer team from Bergamo wins the Champions League match against Valencia 4 to 1 in front of 44,000 fans from Italy (2,000 from Spain). The mass transport from Bergamo to Milan and return, hours of shouting as well as the following festivities in innumerable bars have been considered by some observers as a coronavirus 'biological bomb'.

Thursday, 20 February

A patient in his 30s tested positive for SARS-CoV-2 and was admitted to the intensive care unit (ICU) in **Codogno** Hospital (Lodi, Lombardy, Italy). The symptomatic patient had visited the hospital the day before but was not tested as he did not meet the suspected case epidemiological criteria (no link with China). His wife, 5 hospital staff, 3 patients and several contacts of the index patients also tested positive to the COVID-19. Over the next 24 hours, the number of reported cases would increase to 36, many without links to the Codogno patient or previously identified positive cases. A first COVID-19 death in a 78-year-old man was also reported. It is the beginning of the Italian epidemic. jamanetwork.com/journals/jama/fullarticle/2763188

Saturday, 22 February

South Korea reports a sudden spike of 20 new cases of coronavirus infection, raising concerns about a potential "super spreader" who has already infected 14 people in a church in the south-eastern city of Daegu.

Sunday, 23 February

Italy confirms 73 new cases, bringing the total to 152, and a third death, making Italy the third country in the world by number of cases, after China and South Korea. A “red zone” area around Codogno is created, isolating 11 municipal areas. Schools are closed.

Venice Carnival is brought to an early close and sports events are suspended in the most-hit Italian regions.

Monday, 24 February

France, Bahrain, Iraq, Kuwait, Afghanistan and Oman report their first cases.

Tuesday, 25 February

A report of a joint WHO mission of 25 international and Chinese experts is presented to the public. The mission travelled to several different Chinese provinces. The most important findings are that the Chinese epidemic peaked and plateaued between the 23rd of January and the 2nd of February and declined steadily thereafter (Table 1).

[https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-\(covid-19\)](https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19))

This was the first sign that the **aggressive use of quarantine** ordered by the Chinese government was the **right thing to do**. Unfortunately, European countries which did not experience the SARS epidemic in 2003, would lose precious time before following the Chinese example.

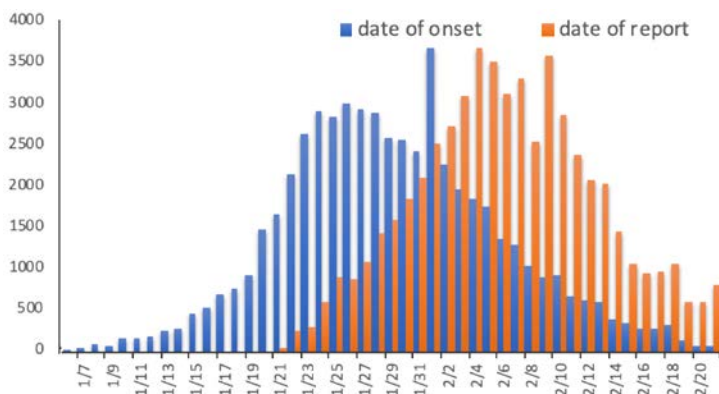


Figure 1. COVID-19 cases in China, January/February 2020. Epidemic curves by symptom onset and date of report on 20 February 2020 for laboratory confirmed COVID-19 cases for all of China. Modified from *Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19)*. 16-24 February 2020. [https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-\(covid-19\)](https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19))

Wednesday, 26 February

A president, fearing for his chances to be re-elected, downplays the threat from the coronavirus pandemic, twittering: “Low Ratings Fake News...are doing everything possible to make the Coronavirus [sic] look as bad as possible, including panicking markets, if possible.”

<https://www.bmj.com/content/368/bmj.m941>

Two days later, the same individual invokes magic: “It’s going to disappear. One day, it’s like a miracle, it will disappear.”

P.S. On 28 March, The Guardian would ask why this person **failed the biggest test of his life**.

Friday, 28 February

A quick look at European cases diagnosed outside of Italy from February 24-27 reveals that 31 of 54 people (57%) had recently travelled to **Northern Italy**. Epidemiologists immediately realize that an unusual situation is building up.

Saturday, 7 March

Official data show that **China's exports** plunged 17.2 percent in the first two months of the year.

Sunday, 8 March

The Italian government led by Prime Minister Giuseppe Conte, deserves credit for instauring the first European lockdown, just two and a half weeks after the first autoctone Italian COVID-19 case was detected. First, strict quarantine measures are imposed on 16 million people in the state of Lombardy and 14 other areas in the north. Two days later, Conte would extend these to the entire country of 60 million people, declaring the Italian territory a "security zone". All people are told to stay at home unless they need to go out for "valid work or family reasons". Schools are closed.

Monday, 9 March

A president on Twitter: "So last year 37,000 Americans died from the common Flu. It averages between 27,000 and 70,000 per year. Nothing is shut down, life & the economy go on. At this moment there are 546 confirmed cases of CoronaVirus, with 22 deaths. Think about that!" ([The Guardian](#))

Iran releases 70,000 prisoners because of the coronavirus outbreak in the country.

Tuesday, 10 March

Xi Jinping tours the city of **Wuhan** and claims a provisional victory in the battle against COVID-19. The last two of 16 temporary hospitals in the city are shut down.

Wednesday, 11 March

With more than 118,000 COVID-19 cases in 114 countries and 4,291 deaths, WHO DG declares the coronavirus outbreak a pandemic.

All schools in and around **Madrid**, from kindergartens to universities, are closed for two weeks.

Thursday, 12 March

Italy closes all shops except grocery stores and pharmacies.

In **Spain**, 70,000 people in Igualada (Barcelona region) and three other municipalities are quarantined for at least 14 days. This is the first time Spain adopts measures of isolation for entire municipalities.

Emmanuel Macron, the **French** president, announces the closure of nurseries, schools and universities from Monday, 16 March. He declares: “One principle guides us to define our actions, it guides us from the start to anticipate this crisis and then to manage it for several weeks, and it must continue to do so: it is **confidence in science**. It is to **listen to those who know**.” Some of his colleagues should have listened, too.

Friday, 13 March

The prime minister of an **ex-EU country** introduces the notion of ‘herd immunity’ as a solution to repeated future episodes of coronavirus epidemics. The shock treatment: accepting that 60% of the population will contract the virus, thus developing a collective immunity and avoiding future coronavirus epidemics. The figures are dire. With a little over 66 million inhabitants, some 40 million people would be infected, 4 to 6 million would become seriously ill, and 2 million would require intensive care. Around 400,000 Britons would die. The prime minister projects that “many more families are going to lose loved ones before their time.”

P.S. Five weeks later, The Guardian would still ask, “[How did Britain get its coronavirus response so wrong?](#)”

Saturday, 14 March

The **Spanish** government puts the whole country into lockdown, telling all people to stay home. Exceptions include buying food or medical supplies, going to hospital, going to work or other emergencies.

The **French** government announces the closure of all “non-essential” public places (bars, restaurants, cafes, cinemas, night-clubs) after midnight. Only food stores, pharmacies, banks, tobacconists, and petrol stations may remain open.

Sunday, 15 March

France calls 47 million voters to the poll. Both government and opposition leaders seem to be in favor of maintaining the municipal elections. Is this a textbook example of unacceptable interference of party politics with the sound management of a deadly epidemic? Future historians will have to investigate.

Monday, 16 March

Ferguson et al. publish a new modelling study on likely UK and US outcomes during the COVID-19 pandemic. In the (unlikely) absence of any control measures or spontaneous changes in individual behaviour, the authors expect a peak in mortality (daily deaths) to occur after approximately 3 months. This would result in 81% of the US population, about 264 million people, contracting the disease. Of those, 2.2 million would die, including 4% to 8% of Americans over age 70. More important, by the second week in April, the demand for critical care beds would be 30 times greater than supply.

The model then analyzes two approaches: mitigation and suppression. In the mitigation scenario, SARS-CoV-2 continues to spread at a slow rate, avoiding a breakdown of hospital systems. In the suppression scenario, extreme social distancing measures and home quarantines would stop the spread of the virus. The study also offers an outlook at the time when strict “Stay at home” measures are lifted. The perspective is grim: the epidemic would bounce back.

France imposes strict confinement measures.

Tuesday, 17 March

Seven million people across the **San Francisco Bay Area** are instructed to “shelter in place” and are prohibited from leaving their homes except for “essential activities” (purchasing food, medicine, and other necessities). Most businesses are closed. The exceptions: grocery stores, pharmacies, restaurants (for takeout and delivery only), hospitals, gas stations, banks.

Thursday, 19 March

For the first time since the beginning of the coronavirus outbreak, there have been **no new cases in Wuhan** and in the Hubei province.

Californian Governor Gavin Newsom orders the entire population of **California** (40 million people) to “stay at home”. Residents can only leave their homes to meet basic needs like buying food, going to the pharmacy or to the doctor, visiting relatives, exercising.

Friday, 20 March

Italy reports 6,000 new cases and 627 deaths in 24 hours.

In **Spain**, the confinement due to the coronavirus reduces crime by 50%.

China reports no new local coronavirus cases for three consecutive days. Restrictions are eased, **normal life resumes**. The entire world now looks at China. Will the virus spread again?

The state of **New York**, now the center of the U.S. epidemic (population: 20 million), declares a general lockdown. Only essential businesses (grocers, restaurants with takeout or delivery, pharmacies, and laundromats) will remain open. Liquor stores? Essential business!

Sunday, 22 March

Byung-Chul Han publishes *La emergencia viral y el mundo de mañana* (El País): “Asian countries are managing this crisis better than the West. While there you work with data and masks, here you react late and borders are opened.”

Monday, 23 March

Finally, too late for many observers, the UK puts in place containment measures. They are less strict than those in Italy, Spain and France.

German Chancellor Angela Merkel self-quarantines after coming into contact with a person who tested positive for coronavirus.

Tuesday, 24 March

Of all reported cases in Spain, 12% are among health care workers.

The Tokyo Olympics are postponed until 2021.

India orders a nationwide lockdown. Globally, three billion people are now in lockdown.

Wednesday, 25 March

After weeks of stringent containment measures, Chinese authorities lift travel restrictions in Hubei province. In order to travel, residents will need the “Green Code” provided by a monitoring system that uses the AliPay app.

A 16-year-old girl dies in the south of Paris from COVID-19. The girl had no previous illnesses.

Thursday, 26 March

America First: the US is now the country with most known coronavirus cases in the world.

For fear of reactivating the epidemic, China bans most foreigners from entering the country.

Friday, 27 March

The [Prime Minister](#) and the Ministre of Health of an ex-EU country tests positive for coronavirus.

The Lancet publishes *COVID-19 and the NHS—"a national scandal"*.

A paper by [McMichael et al.](#) describes a 33% case fatality rate for SARS-CoV-2 infected residents of a long-term care facility in King County, Washington, US.

Sunday, 29 March

[The Guardian](#) and the [Boston Globe](#) ask who might have blood on their hands in the current pandemic. The evolution of the US epidemic is being described as the [worst intelligence failure in US history](#).

Monday, 30 March

[Flaxman S et al.](#) from the Imperial College COVID-19 Response Team publish new data on the possibly true number of infected people in **11 European countries**. Their model suggests that as of 28 March, in Italy and Spain, 5.9 million and 7 million people could have been infected, respectively (see [Table](#) online). Germany, Austria, Denmark and Norway would have the lowest infection rates (proportion of the population infected). These data suggest that the **mortality of COVID-19 infection** in Italy could be in the range of 0.4% (0.16%-1.2%).

Moscow and **Lagos** (21 million inhabitants) go into lockdown.

The COVID-19 crisis causes some **East European political leaders** to consider legislation giving them extraordinary powers. In one case, a law was passed extending a state of emergency indefinitely.

SARS-CoV-2 is spreading aboard the aircraft carrier USS *Theodore Roosevelt*. The ship's commanding officer, Captain Brett Crozier,

sends an email to three admirals in his chain of command, recommending that he be given permission to evacuate all non-essential sailors, to quarantine known COVID-19 cases, and sanitize the ship. “We are not at war. [Sailors do not need to die](#),” writes Crozier in his four-page memo. The letter leaks to the media and generates several headlines. Three days later, 2 April, Captain Crozier is sacked.

Later, testing of 94% of the crew of roughly 4,800 people would reveal around 600 sailors infected, a majority of whom, around 350, were asymptomatic.

Wednesday, 1 April

The United Nations chief warns that the coronavirus pandemic presents the world’s “worst crisis” since World War II.

Thursday, 2 April

Worldwide more than one million cases are reported. The true number is probably much higher (see the [Flaxman paper](#) on 30 March).

European newspapers run articles about why Germany has so few deaths from COVID-19.

Friday, 3 April

Some economists warn that [unemployment](#) could surpass the levels reached during the [Great Depression in the 1930s](#). The good news: almost all governments rate saving tens or hundreds of thousands of lives higher than avoiding a massive economic recession. Has humanity become more human?

Le Monde, the most influential French newspaper, points to a more [mundane side effect](#) of the epidemic. As hairdressers are forbidden to work, colors and cuts will degrade. The newspaper

predicts that “after two months, 90% of blondes will have disappeared from the face of the Earth”.

Saturday, 4 April

In Europe, there are signs of hope. In Italy, the number of people treated in intensive care units decreases for the first time since the beginning of the epidemic.

In France, 6,800 patients are treated in intensive care units. More than 500 of these have been evacuated to hospitals from epidemic hotspots like Alsace and the Greater Paris area to regions with fewer COVID-19 cases. Specially adapted TGV high-speed trains and aircraft have been employed.

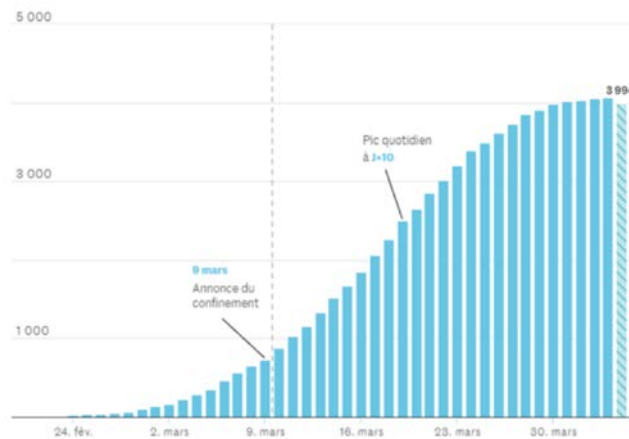


Figure 2. Patients treated in intensive care units in Italy. For the first time since the beginning of the epidemic, the number decreases on 4 April.
Source: [Le Monde](#)

Lombardy decides that as of Sunday 5 April, people must wear masks or scarves. Supermarkets must provide gloves and hydroalcoholic gel to their customers.

An Italian politician, less penetrable to scientific reasoning on a par with some of his colleagues in the US and Brazil, asks for churches to be open on Easter (12 April), declaring that “science alone is not enough: the good God is also needed”. *Heureux les simples d’esprit*, as the French would say.

Sunday, 5 April

The US surgeon general warns the country that it will face a “[Pearl Harbor moment](#)” in the next week.

US is the new epicenter of the COVID-19 epidemic. By the time of this writing (5 April), more than 300,000 cases and almost 10,000 deaths were reported. Almost half were reported from New York and New Jersey.

Tuesday, 7 April

Air quality improves over Italy, the UK and Germany, with falling levels of carbon dioxide and nitrogen dioxide. Will a retrospective analysis of the current lockdown reveal fewer cases of asthma, heart attacks and lung disease?

Wednesday, 8 April

Japan declares a state of emergency, Singapore orders a partial lockdown.

In Wuhan people are allowed to travel for the first time since the city was sealed off 76 days ago.

The Guardian publishes a well-documented timeline: “[Coronavirus: 100 days that changed the world.](#)”

Thursday, 9 April

EU finance ministers agree to a common emergency plan to limit the impact of the coronavirus pandemic on the European economy. The Eurogroup reaches a deal on a [response plan worth more than €500 billion](#) for countries hit hardest by the epidemic.

Passenger air travel has decreased by up to 95%. How many of the 700 airlines will [survive](#) the next few months? Will the current interruption of global air travel [shape our future travel behaviors](#)?

The epidemic is devastating the US economy. More than 16 million Americans have submitted unemployment claims in the past three weeks.

Friday, 10 April

COVID-19 treatment for one dollar a day? [British, American and Australian researchers](#) estimate that it could indeed cost only between 1 and 29 dollars per treatment and per patient.

Message from your mobile phone: “You have been in contact with someone positive for coronavirus.” Google and Apple announce that they are **building a coronavirus tracking system into iOS and Android**. The joint effort would enable the use of Bluetooth technology to establish a voluntary contact-tracing network. Official apps from public health authorities would get extensive access to data kept on phones that have been in close proximity with each other (George Orwell is turning over in his grave). If users report that they’ve been diagnosed with COVID-19, the system would alert people if they were in close contact with the infected person.

Spain discovers *COVID Reference*. Within 24 hours, more than 15,000 people download the [PDF of the Spanish edition](#). The only explanation: a huge media platform displayed the link of our book. Does anyone know who did it?

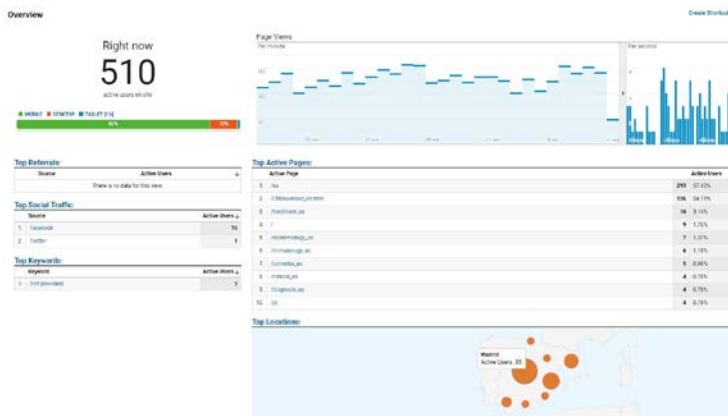


Figure 3. Google Analytics data for www.CovidReference.com on 10 April. At one moment, more than 500 people, mostly from Spain, were visiting the website simultaneously.

Saturday, 11 April

More than **400 of 700 long-term care facilities** (EHPAD in French, *Etablissement d'Hébergement pour Personnes Agées Dépendantes*) in the greater Paris region (pop. – 10 million) have COVID-19 cases.

In Italy, **110 doctors** and about 30 other hospital workers have died from COVID-19, half of them nurses.

Sunday, 12 April

Easter 2020. Italy reports 361 new deaths, the lowest number in 25 days while Spain reports 603 deaths, down more than 30% from a high 10 days before.

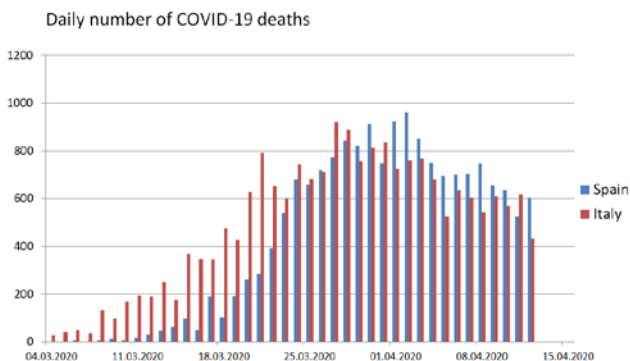


Figure 4. Daily number of COVID-19 deaths in Italy (red) and Spain (blue).

The United Kingdom records its highest daily death toll of almost 1,000. The number of reported COVID-19-linked fatalities now exceeds 10,000. As in many other countries, the true numbers may be slightly higher due to underreporting of [people dying in care homes](#).

The number of COVID-19-related deaths in the United States passes 22,000, while the number of cases tops 500,000. In New York there are signs that the pandemic could be nearing its peak.

Monday, 13 April

The COVID-19 pandemic exposes **bad governance**, not only in Brazil. The French newspaper *Le Monde* reveals the ingredients: denial of reality, search for a scapegoat, omnipresence in the media, eviction of discordant voices, political approach, isolationism and short-term vision in the face of the greatest health challenge in recent decades. [The culprit?](#)

Emmanuel Macron announces a **month-long extension to France's lockdown**. Only on Monday, May 11, nurseries,

primary and high schools would gradually reopen, but not higher education. Cafés, restaurants, hotels, cinemas and other leisure activities would continue to remain closed after May 11.

Tuesday, 14 April

Austria is the first European country to **relax lockdown measures**. It opens up car and bicycle workshops, car washes, shops for building materials, iron and wood, DIY and garden centers (regardless of size) as well as smaller dealers with a customer area under 400 square meters. These shops must ensure that there is only one customer per 20 square meters. In Vienna alone, 4,600 shops are allowed to open today. Opening times are limited to 7.40 a.m. to 7 p.m. The roadmap for the coming weeks and months:

- 1 May: All stores, shopping malls and hairdressers reopen (see also the April 3 entry, page 354).
- 15 May: Other services such as restaurants and hotels remain closed at least until mid-May.
- 15 May or later: Possible re-opening of classes in schools.
- July: possible – but improbable – organization of events of all sorts (sport, music, theater, cinema etc.).

There is a general obligation to wear a mask when shopping and on public transport.

The International Monetary Fund (IMF) forecasts a **contraction of 3% of the planet's GDP in 2020**. The possibility of an even more brutal fall in 2021 is not excluded. The possibly worst economic downturn since the Great Depression in 1929 will not spare any continent. In a recession like no other in peacetime for nearly a century, the countries of the eurozone, the United Kingdom and the United States might see a contraction in activi-

ty of between 5.9% and 7.5%. China's economy is expected to grow by about 1%.

US: The CDC ([Centers for Disease Control and Prevention](#)) reports that more than 9,000 health care workers contracted COVID-19 as and at least 27 died. The median age was 42 years, and 73% were female. Deaths most frequently occurred in HCP aged ≥ 65 years.

Wednesday, 15 April

[Philip Anfinrud and Valentyn Stadnytsky](#) from the National Institutes of Health, Bethesda, report a laser light-scattering experiment in which speech-generated droplets and their trajectories were visualized. They find that when a test person says, "stay healthy," numerous droplets ranging from 20 to 500 μm are generated. When the same phrase is uttered three times through a slightly damp washcloth over the speaker's mouth, the flash (droplet) count remains close to the background level. The video supports the recommendation of wearing face masks in public. The authors also found that the number of flashes (droplets) increased with the loudness of speech. The new message for billions of people caught in the COVID-19 epidemic: lower your voice!

Friday, 17 April

Luiz Inácio Lula da Silva, the former Brazilian president says that the current president is leading Brazil to "the slaughterhouse" with his irresponsible handling of coronavirus. In an [interview with The Guardian](#), Lula says that Brazil's "troglodyte" leader risks repeating the devastating scenes playing out in Ecuador where families have to dump their loved ones' corpses in the streets.

On the French aircraft carrier **Charles-de-Gaulle**, a massive epidemic is. Among the 1760 sailors, 1,046 (59%) are positive for

SARS-CoV-2, 500 (28%) present symptoms, 24 (1.3%) sailors are hospitalized, 8 on oxygen therapy and one in intensive care.

Saturday, 18 April

Chancellor Angela Merkel makes a television speech, her first in over 14 years in office. She describes the coronavirus crisis “as the greatest challenge since the Second World War” and exhorts the Germans: “It is serious. Take it seriously.”

Care England, Britain’s largest representative body for care homes, suggests that up to 7,500 residents may have died of COVID-19. This would be higher than the 1,400 deaths estimated by the government.

In [Catalunya alone](#), some 6,615 hospital professionals and another 5,934 in old age care homes are also suspected of having or been diagnosed with COVID-19.

Sunday, 19 April

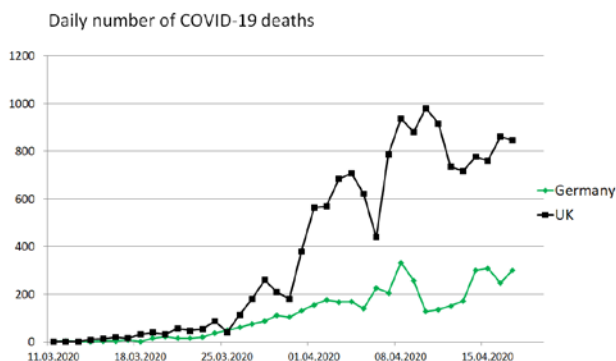


Figure 5. Daily number of COVID-19 deaths in Germany (green) and the United Kingdom (black).

Air traffic in Europe has plummeted more than 95% as nicely shown by this YouTube video by The Guardian: <https://www.youtube.com/watch?v=lOVP2o3c4Gw>

Monday, 20 April

For the first time in history, the West Texas Intermediate (WTI), the benchmark price for US oil, drops below \$0. On certain specific contracts, it plunged down to minus 37 US dollars (-34 euros). After nearly two months of continuous collapse of the oil market, this paradoxical situation is the result of the COVID-19 pandemic which caused demand to fall by 30%. As oil wells continue to produce, there is no place to store the oil and investors are ready to pay to get rid of it.

Germany's **Oktoberfest is cancelled**. The iconic beer festival, colloquially known as *Die Wiesn* or "the meadow", attracts around 6 million visitors from around the world. It runs for more than two weeks (September/October) in packed tents with long wooden tables, where people celebrate traditional food, dancing, beer and clothing. The loss for the city of Munich is estimated to be around one billion euros.

Tuesday, 21 April

The Spanish newspaper El País publishes an **intelligible overview** of the battle between SARS-CoV-2 and the human body: "Así es la lucha entre el sistema inmune y el coronavirus." ¡Fantástico!

Cancer Research UK reports that every week, 2,300 people with cancer symptoms are no longer examined. Screening examinations for breast and uterine cancer of over 200,000 women per week have been cancelled. According to **The British Heart Foundation**, 50 percent fewer people suspected of having a heart attack attended hospital emergency rooms in March. A 50% drop would be "equivalent to approximately 5000 of the expected people every month, or more than 1100 people every week, with

possible heart attack symptoms not being seen in emergency departments.” Will we discover a hidden epidemic of COVID-19-related morbidity and mortality with millions of people dying not from coronavirus, but from other, actually treatable diseases?

Thursday, 23 April

Pandemic hilarity, as a president known for his poor science record stammers speculations about “injecting” “disinfectant” to cure COVID-19.

Sunday, 26 April

The city of Wuhan announces that all remaining COVID-19 cases have been discharged from the hospitals.

Monday, 27 April

Are genes determining coronavirus symptoms? After studying 2,633 identical and fraternal twins who were diagnosed with COVID-19, a group from King’s College London reports that COVID-19 symptoms appear to be 50% genetic (fever, diarrhea, delirium and loss of taste and smell)¹⁵. It is as yet unclear whether and to what extent [reported deaths of identical twins](#) can be attributed to genetic factors.

Friday, 1 May

A new SARS-CoV-2 test could be able to identify virus carriers before they are infectious, according to a report by [The Guardian](#). The blood-based test would be able to detect the virus’s pres-

¹⁵ Williams FMK et al. **Self-reported symptoms of covid-19 including symptoms most predictive of SARS-CoV-2 infection, are heritable.** MedRxiv 27 April (accessed 8 May 2020). Abstract: <https://www.medrxiv.org/content/10.1101/2020.04.22.20072124v2>

ence as early as 24 hours after infection – before people show symptoms and several days before a carrier is considered capable of spreading it to other people.

Sunday 3 May

Roche gets US Food and Drug Administration emergency use approval for its antibody test, **Elecsys** Anti-SARS-CoV-2, which has a specificity rate of about 99.8% and a sensitivity rate of 100%.

Monday, 4 May

Italy is cautiously easing lockdown measures. People can go jogging but may not go to the beach; they may surf but now swim; and they can visit 6th grade relatives, but not friends, lovers or mistresses.

A French hospital that retested old samples from pneumonia patients discovers that it treated a man with the coronavirus **as early as 27 December**, a month before the French government confirmed its first cases.

Researchers from Bonn University, Germany, report a **sero-epidemiological study** of 919 people from Gangelt, a small German town which was exposed to a super-spreading event (carnival festivities). 15.5% were infected, with an estimated infection fatality rate of 0.36%. 22% of infected individuals were asymptomatic.

Tuesday, 5 May

Neil Ferguson, epidemiologist at the Imperial College, resigns his post as member of the British government's Scientific Advisory Group for Emergencies (SAGE) over an “error of judgement”. A newspaper had reported that he did not respect the rules of con-

finement (which he himself had contributed to establishing!) by receiving at least twice a 38-year-old woman at his home.

Anthony Fauci, the director of the United States National Institute of Allergy and Infectious Diseases, says that there is no scientific evidence to back the theory that the coronavirus was made in a Chinese laboratory or leaked from a laboratory after being brought in from the wild ([CGTN](#)).

Wednesday, 6 May

The official COVID-19 death toll in the UK exceeds 30,000.

Thursday, 7 May

According to data released by the US Department of Labor, more than 33 million Americans have filed for initial jobless claims. This corresponds roughly to 21% of the March labor force.

Only 15 countries in the world have not officially reported a case of COVID-19 to WHO, namely: North Korea, Turkmenistan, Kiribati, Marshall Islands, Micronesia, Samoa, Solomon Island, Tonga, Tuvalu, Vanuatu, Cook Island, Nauru, Niue, Palau and Lesotho. (We know North Korea is cheating, and Turkmenistan and Lesotho cannot deny for long... It's a true pandemic!)

According to figures by the [Office of National Statistics](#), black people are more than four times more likely to die from COVID-19 than white people.

Friday, 8 May 2020

After pipedreams (German: Hirngespinnste; French: élucubrations; Italian: visioni; Spanish: fantasías) about hydroxychloroquine and injecting disinfectants, today is the day where COVID-19 will “go away without vaccine”. The sad developments of the coronavirus pandemic have now accumulated sufficient evidence that the individual doesn't believe himself what he is saying. The

carefully timed and well-orchestrated ungrammatical utterings just obey one supreme life mission: continue staying in the news. Alas, there is an even more tragic aspect to the drama: Why on Earth do the world's media insist on talking about this individual? Why can't we read the news without seeing his face every single day? Why couldn't we simply *totschweigen* him? (*Totschweigen* is a superbly descriptive German verb: 1. *tot* dead; 2. *schweigen* to be silent; 3. *totschweigen* make someone dead silent – English: to hush up; French: passer sous silence; Italian: fare come se non esistesse; Portuguese: não falar em alguém.)

Today, we make a funereal promise: we'll never talk about the individual again, not even on the day he dies.

Sunday, 10 May

Italians are looking on aghast at the UK's coronavirus response, says [The Guardian](#). Is it really no accident that Britain and America are the world's [biggest coronavirus losers](#)?

Everything you always wanted to know about false negatives and false positives* (*but were afraid to ask) is now summarized in [10 steps to understand COVID-19 antibodies](#). The colors will help you memorize true and false negatives and positives.

Spain's best newspaper El País publishes '[ccu ccg ccg gca – The 12 letters that changed the world](#).' (If you read Spanish, take a look.)

Monday, 11 May

France eases lockdown restrictions among a sense of uncertainty. The newspaper [Le Monde reports](#) that according to official figures 8,674 new positive tests for SARS-CoV-2 were registered between May 1 and 9. Epidemiologist Daniel Lévy-Bruhl, head of the respiratory infections unit of Santé Publique France (Public Health France) estimates that the real figures are probably twice or three times as high (3,000 to 4,000 new infections each day) –

despite barrier gestures, social distancing and general confinement.

Tuesday, 12 May

The MMWR publish a report about a [high SARS-CoV-2 attack rate following exposure at a choir practice](#).

Wednesday, 13 May

There is evidence that **China** is **censoring COVID Reference**. Google Analytics data of two dozen websites, both medical ([Amedeo](#), [Free Medical Journals](#), [FreeBooks4Doctors](#)) and non-medical ([TheWordBrain](#), [Ear2Memory](#), [GigaSardinian](#), [GigaMartinique](#), [SardoXSardi](#), [Polish Yiddish](#) and [ItalianWithElisa](#), among others) show that by number of visitors, China was always among the *Top 10 countries*, generating between 3.3% and 14.8% of website traffic (see <https://covidreference.com/censorship>).

Not so with COVID Reference. Six weeks after the launch of COVID Reference, China is 27th, after Paraguay, accounting for 0.39% of global traffic. Is someone standing on the data line between COVID Reference and China (Figure 6)?

25.	 Costa Rica	790 (0.42%)
26.	 Paraguay	744 (0.40%)
27.	 China	727 (0.39%)
28.	 Netherlands	716 (0.38%)
29.	 Russia	613 (0.33%)

Figure 6. Google Analytics data for www.CovidReference.com on 13 May. Six weeks after the launch of COVID Reference, China is 27th, after Paraguay and right before the Netherlands and Russia.

Friday, 15 May

In a memorable [blog entry for the British Medical Journal](#), Paul Garner, professor of infectious diseases at Liverpool School of Tropical Medicine, discusses his COVID-19 experience as having “been through a roller coaster of ill health, extreme emotions, and utter exhaustion”.

A [video experiment](#) using black light and a fluorescent substance demonstrates how quickly germs can be spread in environments such as restaurant buffets and cruise ships: www.youtube.com/watch?v=kGQEuuv9R6E.

Saturday, 16 May

A new highly transmissible and potentially deadly virus is detected in Germany: **SADS**, Severe Acute Dementia Syndrome. The new syndrome manifests as an irrepressible desire to ignore the danger of COVID-19. In several German cities, an improbable alliance takes to the streets – left- and right-wing extremists, antisemites, conspiracy theorists and anti-vaxxers –, claiming the right to live and to die without social distancing and face masks. The German Government immediately informs WHO.

Monday, 18 May

Merkel and Macron announce a 500,000 million euro aid plan for the reconstruction of Europe ([El País](#)).

Moderna announces that its experimental vaccine mRNA-1273 has generated antibodies in eight healthy volunteers ages 18 to 55. The levels of neutralizing antibodies matched or exceeded the levels found in patients who had recovered from SARS-CoV-2 infection ([The Guardian](#)).

Wednesday, 20 May

After an outbreak of coronavirus, Chinese authorities seal off the city of Shulan, a city of 700,000 close to Russian border, imposing measures similar to those used in Wuhan ([The Guardian](#)).

Google and Apple release their Exposure Notification System to notify users of coronavirus exposure: <https://www.google.com/covid19/exposurenotifications>.

We discover a website which shows where infected people in Hong Kong are at all times: <https://chp-dashboard.geodata.gov.hk/covid-19/en.html> (Figure 7). There is no doubt that the tighter you control the infected, the less restriction you have to impose on the uninfected. In Europe, strict measures such as those adopted in Hong Kong and South Korea are currently not compatible with existing legislation about privacy.

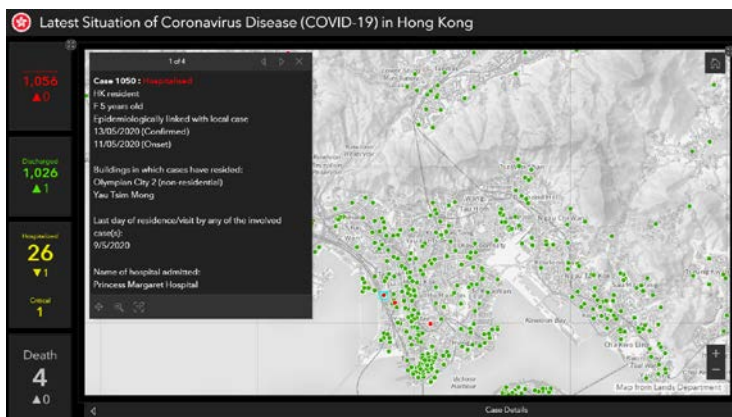


Figure 7. Screenshot of the “Latest Situation of Coronavirus Disease (COVID-19) in Hong Kong”, <https://chp-dashboard.geodata.gov.hk/covid-19/en.html>.

Thursday, 21 May

The Centers for Disease Control and Prevention (CDC) informs that rats rely on the food and waste generated by restaurants and other commercial establishments, the closures of which have led to food shortage among rodents, especially in dense commercial areas. CDC warns of [unusual or aggressive rodent behavior](#).

Will SARS-CoV-2 seal the fate of the Airbus A380? Air France chooses to end the operations of the aircraft, judged to be too expensive, too polluting and not profitable enough ([Le Monde](#)).

Friday, 22 May

Zhu et al. publish *Safety, Tolerability, and Immunogenicity of a Recombinant Adenovirus type-5 Vectored COVID-19 Vaccine*.

Fafi-Kremer 2020 et al. pre-publish *Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France*, reporting that neutralizing antibodies against SARS-CoV-2 were detected in virtually all hospital staff (n=160) sampled from 13 days after the onset of COVID-19 symptoms (see also [Le Monde](#)).

Saturday, 23 May

In Lower Saxony, Germany, 50 people are in quarantine after an outbreak in a restaurant ([Der Spiegel](#)).

In Frankfurt, Germany, authorities report more than 40 people infected with SARS-CoV-2 after a religious service ([Der Spiegel](#)).

Wednesday, 27 May

Colombian designers prepare cardboard hospital beds that double as coffins ([The Guardian](#)).

Andrzej Krauze publishes a [cartoon](#) on the fallout from the COVID-19 pandemic.

Sunday, 31 May

More than 50 million people across the US could go hungry without help from food banks or other aid ([Feeding America](#)).

Wednesday, 3 June

In the hope of saving its tourist industry, Italy reopens its borders.

Tuesday, 4 June

The Lancet *makes one of the biggest retractions in modern history* (The Guardian).

Friday, 5 June

The chief investigators of the RECOVERY trial report that there is [no clinical benefit from use of hydroxychloroquine in hospitalised patients with COVID-19](#).

Saturday, 6 June

The Guardian reports that [nearly 600 US health workers have died of COVID-19](#).

Sunday, 7 June

Three super-spreading events in an office, a restaurant and a bus show how easily SARS-CoV-2 can be spread over distances of more than 1 meter. The feature by [El País](#) is worth taking a look, even if you don't understand Spanish: <https://elpais.com/ciencia/2020-06-06/radiografia-de-tres-brotes-asi-se-contagiaron-y-asi-podemos-evitarlo.html>.

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